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UNROLLING OF LEAVES OF MUSA SAPIENTUM AND SOME RELATED PLANTS AND THEIR REACTIONS TO ENVIRONMENTAL ARIDITY[1]

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(WITH TWENTY-THREE FIGURES)

Introduction

In two previous publications the anatomy (5) and the development and morphology (6) of the leaf of the banana have been discussed. The present paper is devoted to a description of the unfurling of the lamina, and of the development of the tissues of the pulvinar band, by the agency of which the lamina halves fold together beneath the midrib in the middle of dry days and spread out again toward evening. For purposes of comparison, I shall discuss the same points in several other genera, representing each of the four families into which the Scitamineae is divided.

The first paper figured and described a peculiar hypertrophy of the cells of the upper water tissue at points where the longitudinal furrows in the surface of the lamina (caused by pressure exerted upon it while still rolled within the false stem) intersect one of the principal veins. At that time I was unable to explain the significance of the enormous anticlinal elongation of these cells, but merely recorded the anatomical peculiarity. During a recent visit to Panama experiments were performed on the mechanism of the unrolling of the lamina which make it clear that hypertrophy of the cells of the water

[1] Botanical contribution from the Johns Hopkins University, no. 109.

tissue at the points in question is the direct result of the difficulty in flattening out these folds when the leaf unrolls.

Since my earlier description of the banana leaf was written, Löv (3) has studied the mechanism of the unfolding of monocotyledonous leaves. In most families of this class the leaf, whether plicate, convolute, involute, or otherwise disposed in vernation, does not expand as a result of the properly modulated or harmonious growth of all of its tissues, but rather through the rapid enlargement of certain more or less specialized cells which up to the time of unfolding have lagged behind the others in development. These cells, which are designated "unfolding cells" (Entfaltungszellen) in the German literature on the subject, and for which I propose the term *expansion cells*, from their rôle in the expansion of the lamina, are of necessity situated on that surface of the leaf which is concave in vernation. In most monocotyledons this appears to be the upper or ventral surface, although in the plicate leaves of palms expansion cells are found alternately on the dorsal and ventral surfaces, as governed by the direction of the folds. Expansion cells may be either epidermal, hypodermal, or more deeply situated. In the Gramineae, Cyperaceae, Juncaceae, Liliaceae, Amaryllidaceae, and Commelinaceae the information at present available indicates that the expansion cells are typically epidermal, although in many cases they are aided by more deeply situated tissues. In the Pandanaceae, Palmae, and Scitamineae the expansion cells are almost always hypodermal (water tissue). The epidermis contributes at most a very subordinate aid in the process of unfolding, but the mesophyll (as distinct from the hypoderm) may in some cases be of substantial help. In the Orchidaceae there is considerable variation in the position of the expansion cells. In the whole order of the Helobiae they are apparently absent, as they are from the Bromeliaceae and the leaves of the Araceae (although present in the spathes of certain species) and from a series of genera in the Amaryllidaceae. In these leaves the unfolding is accomplished by the coordinate development of all of the tissues, the progressively increasing growth of all cells from the lower surface to the upper.

In this rapid survey it has been necessary to confine statements to the broadest generalizations; the numerous exceptions may be found

in Löv's memoir. In some species great variation is found even between different regions of the same leaf. Thus in the upper portion of the leaf of *Homeria collina* the expansion cells are developed from the hypoderm and the mesophyll, while in the basal portion the epidermis also contributes to their number.

In regard to their distribution over the surface of the leaf, considerable variation exists. In the Commelinaceae, for example, the epidermis over the whole of the upper surface consists of expansion cells. In many grasses, palms, and other leaves with particular lines of folding, the expansion cells are more or less restricted to definite longitudinal series at these places.

Microscopically expansion cells are characterized by their thin, generally unsuberized walls and large clear lumina. Tannin, crystals, oil bodies, and leucoplasts are seldom present. Chlorophyll is present only in expansion cells which develop from the mesophyll, and even in these it typically occurs only in small amounts.

The expansion cells of the grasses, which generally contrast sharply with the neighboring epidermal cells, were apparently the first to receive the attention of botanists, and were described by DUVAL-JOUVE (1) in 1875. Later TSCHIRCH (7) devoted a special study to the inrolling of the leaves of xerophytic grasses, which is at least in part effected by their expansion cells. The expansion cells of grasses are apparently the only ones mentioned in the text-books, and then only in regard to the opening and closing of these leaves in response to wetting and drying, rather than in relation to their primary function in the unfolding of the young leaf. RUDOLPH (4) has described the mechanism of unfolding of the leaves of palms.

Unrolling of banana leaf[2]

At the moment the tip of the young leaf emerges from the top of the false stem, the lamina is practically full-grown. After tearing away the leaf sheaths, the tightly rolled lamina may be unfurled without

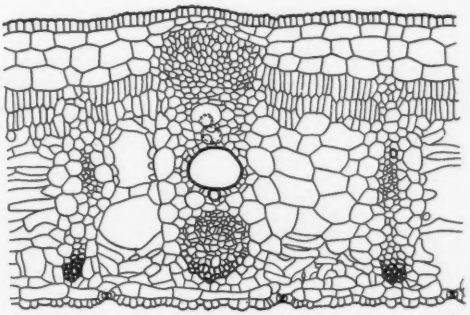
[2] The descriptions and drawings refer to apparently undescribed varieties of the subspecies *seminifera* of *M. sapientum* introduced to Almirante, Panama, from the Philippine Islands under various native names, but agreeing so closely in vegetative characters as to be indistinguishable from one another. The varieties of the banana are legion, and their names even more so, but there seems to be essential agreement within the species in anatomical characters, and the present account gives a description of the typical common cultivated varieties.

difficulty. The tissues, except perhaps at the extreme tip, are still ivory white, and the upper surface of the two sides of the lamina is perfectly smooth, without a trace of the transverse ribs so conspicu-

FIG. 1.—Partially expanded lamina of young banana plant (note prominent transverse ribs and reflexed position of sides of lamina at apex).

ous in the mature leaf (fig. 1). These ribs are not primarily places of greater thickness in the lamina, but merely corrugations, the result of the upward curvature of the tissues on either side of the principal veins, so that the latter lie above the general surface of the leaf. On the lower side each ridge is represented by a furrow. The ribs are

formed as the leaf unrolls. They become apparent first at the apex of the left half of the lamina, which is always outermost in the convolute vernation. From this point their formation proceeds basally



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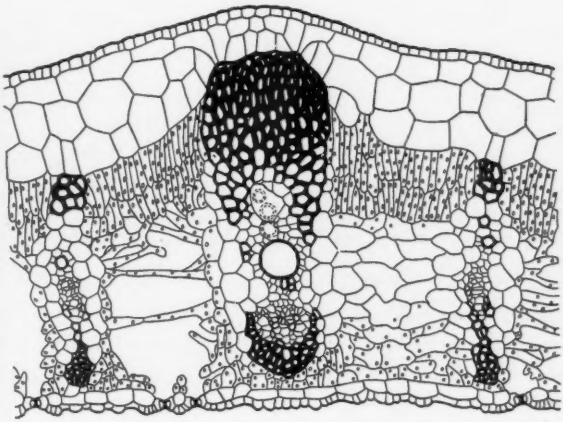

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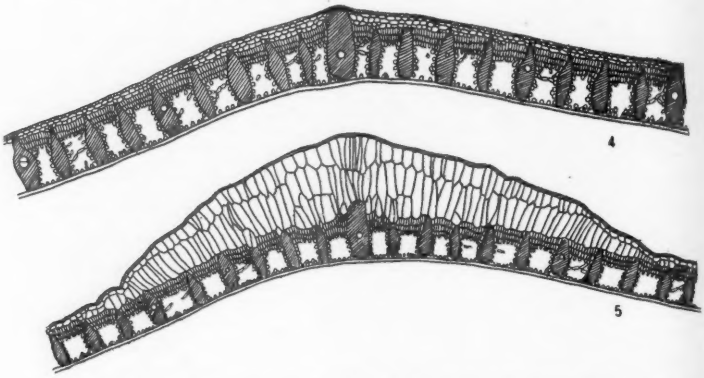
FIGS. 2, 3.—Fig. 2, transverse section through principal and two subordinate veins of lamina not yet unrolling; fig. 3, same, from mature lamina; ×135.

and to the right, keeping pace with the emergence of the leaf, which is effected by rapid elongation of the sheath, particularly its basal portion. Thus the regions of the lamina which are the first physically able to unroll are the first to show the formation of ribs.

Figs. 2 and 3 illustrate the alterations which occur in the cells themselves. Fig. 2 shows a cross-section through principal and subordinate veins of a large leaf just appearing above the false stem, but from near the center of the length of that leaf, and hence from tissues still far down the false stem. Fig. 3 was drawn by camera lucida on the same scale as the preceding, and gives the appearance of the corresponding portion of a mature expanded leaf. Certain evident changes have taken place during the interval between the stages represented by the two drawings, the most conspicuous of which are: (1) the



FIGS. 4, 5.—Fig. 4, transverse section through principal and neighboring subordinate veins of normally expanded lamina; fig. 5, same, from lamina bound 10 days; semi-diagrammatic, water tissue drawn with camera lucida; vascular bundles shaded; ×24.

great enlargement of the cells of the water tissue lying above and on either side of the principal vein; (2) the anticlinal elongation and thickening of the walls of the fibers on the upper side of the principal vein and the thickening of those above the subordinate veins. These cross-sections do not extend far enough laterally to show that the principal vein has been raised above the general surface of the lamina (fig. 4). The changes on the lower side are much less conspicuous. The fibers already show considerable thickening in fig. 2, while those above the xylem are extremely thin walled. The palisade cells have experienced a certain amount of elongation, and the upper epidermal cells have enlarged considerably, but both changes are overshadowed by those of the upper fibrous bundle and the water tissue. It is

evident that the upper portion of the leaf lags behind the lower in its rapidity of differentiation until the moment of unrolling, at which time its development, in relation to a particular function, is greatly accelerated.

Lignification of the fibers lying on the lower side of the vascular bundles of the lamina proceeds *pari passu* with the unfurling of the lamina. The first trace of lignification is evident in the upper left corner shortly before the coils begin to loosen. Thence it proceeds basally and to the right, and lignification of any particular portion of the lamina has usually at least begun by the time that portion begins to unfurl. The production of chlorophyll spreads over the leaf in the same manner, usually keeping a step ahead of the former process, and suggests that the incidence of light is responsible for the initiation of lignification. The same is true in regard to lignification of the hypodermal cells beneath the outer surface of the sheaths (5). The walls of the fibers above the bundles, which never become lignified but at maturity give the reactions of suberized membranes, are at most very slightly thickened during the process of unfurling, and gradually increase in thickness after the leaf has expanded, until finally they are as thick as those of the dorsally situated fibers.

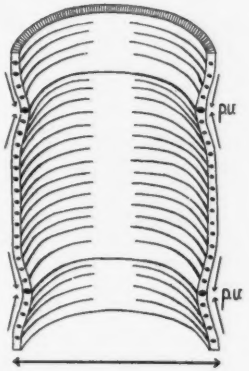
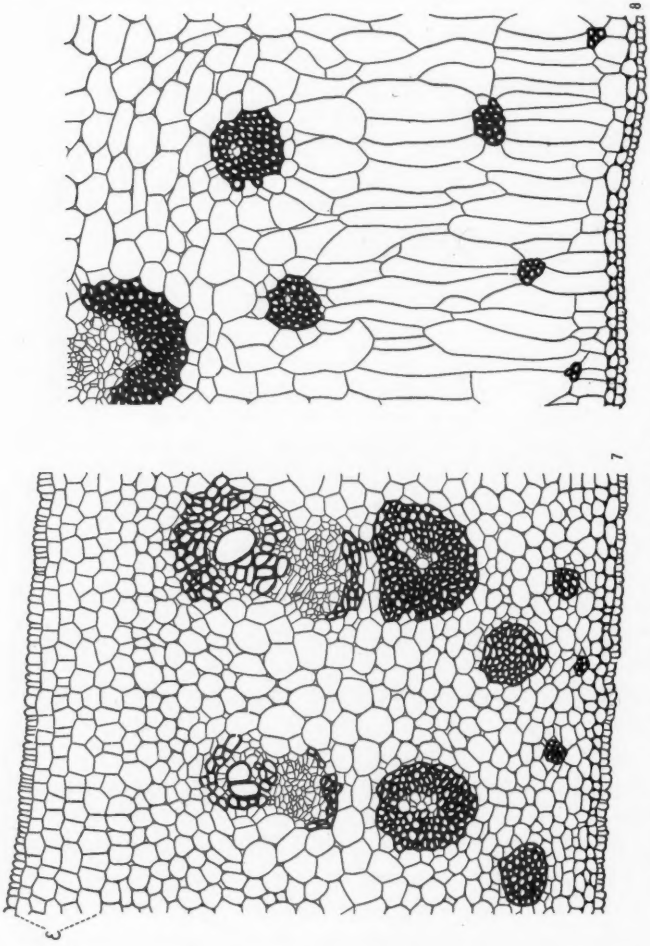


FIG. 6.—Illustrating method of leaf unrolling; arrows show direction of forces resulting from enlargement of expansion cells; *pv*, principal vein.

The great enlargement of the turgid cells of the upper water tissue above and particularly on either side of the principal veins results in pushing these veins above the general surface of the leaf. Since the upper surface is concave in vernation they are forced inward, and occupy, throughout the helix, a curvature of smaller radius than immediately adjacent tissues of the lamina (fig. 6); hence the whole principal vein is thrown into a state of compression in relation to the subordinate veins and the intermediate tissue. The turgid cells of the upper water tissue are most affected by this change, and eventu-

ally occupy a position enabling them to exert their expansive force to the greatest mechanical advantage. The lower side of the leaf,

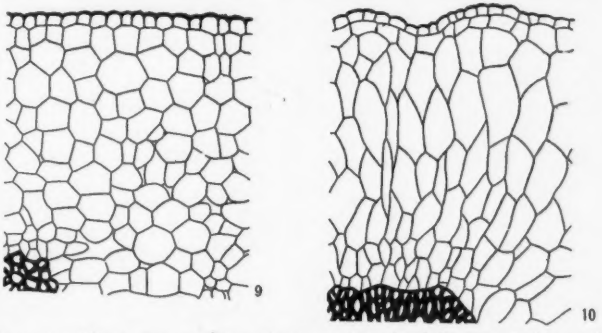


FIGS. 7, 8.—Fig. 7, portion of cross-section of pulvinar band of leaf just appearing from false stem: *e*, expansion cells; fig. 8, lower portion of cross-section of pulvinar band of mature leaf, showing prismatic cells; ×112.

which contains fibers already showing lignification, is relatively resistant to stretching, while the entire upper portion, the fibers in which are still thin walled, is much more easily stretched. The diver-

gent behavior of these two sets of fibers is accordingly of great importance in the unfurling of the leaf.

Meanwhile certain changes are occurring in the tissues lying immediately adjacent to the midrib, which have been called the pulvinar bands (**5**). These bands differ from the remainder of the lamina, among other things, by the absence of lacunae. Were the two lamina halves merely to flatten out without the help of the pulvinar bands they would stand above the midrib with their upper faces together and parallel (**5**, fig. 8). It is the primary function of the pulvinar bands to bend out the lamina halves so that they come to lie



FIGS. 9, 10.—Fig. 9, upper portion of cross-section of pulvinar band of normally unfolded leaf, showing expansion cells (cf. fig. 7*e*); fig. 10, same, from leaf bound 23 days, showing hypertrophy of expansion cells; ×112.

in a plane. Fig. 7 represents a section through the pulvinar band of a large leaf shortly before unfurling. The section was cut transverse to the midrib and parallel to the veins of the lamina. The fibers have already become thick walled (here they rarely become lignified). The cells of the upper water tissue, 4–5 layers deep, are still immature, and as the leaf unfurls they enlarge to the proportions shown in fig. 9, a section through the corresponding region of a mature expanded leaf. By their great enlargement and turgescence these cells bend out the lamina halves along the sides of the midrib, until they actually overstep the degree of flexion necessary to flatten out the leaf and are inclined backward (fig. 1, upper half of unfurling leaf). The process by which they are brought back to position is discussed in a later section of this paper.

Effect of preventing unrolling

By binding the apex of the leaf with soft twine when it first appears, and then continuing to wrap the twine basally as successive portions emerge, over a period of several days, it is possible to prevent unrolling of the lamina without injuring it. By this interference all of the growth processes which effect expansion of the leaf are greatly exaggerated.

By leaving the leaf bound for a week after it has completely emerged, striking results are obtained. When the cord is removed the left half slowly but visibly unrolls, and then begins to roll inward from the margin with the lower side concave (fig. 12), in just the opposite direction from vernation. The principal veins are marked by great welts which are lighter in color than the remainder of the leaf and are hard and turgid to the touch. The strongest of the subordinate veins, one of which lies between each pair of principal veins, are also sometimes swollen, but always to a much less degree, and not continuously. The leaf is much more strongly ribbed than normally. Microscopical examination reveals that the swollen appearance of the principal veins is caused by the great hypertrophy[3] of the cells of the upper water tissue, which elongate anticlinally, the only way they are free to grow. This hypertrophy extends over a variable number of the adjacent subordinate veins, but at its lateral margins the swollen portion of the water tissue is sharply delimited from the cells of normal appearance which occupy most of the interval between the principal veins (fig. 5).

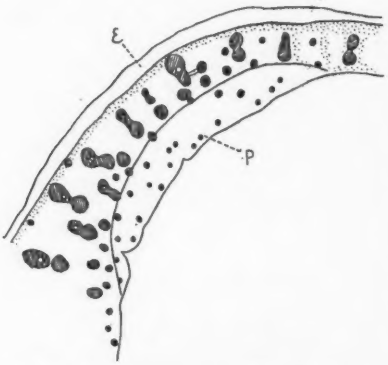


FIG. 11.—Diagrammatic cross-section through pulvinar band of normally expanded leaf, showing expansion cells (*e*) and prismatic cells (*p*); fibrovascular bundles shaded; ×11.

[3] The term hypertrophy is used merely to denote an enlargement of the cells of the upper water tissue, caused by experimental procedure, which is conspicuously above the normal.

Sometimes the tension set up by these enormously swollen cells is so great that the lamina is split inward from the left margin. At such places, or in strips torn by hand from the leaf, the torn margins roll inward over the lower surface, indicating that pressure of the hypertrophied water tissue tends to depress the portion of the lamina be-

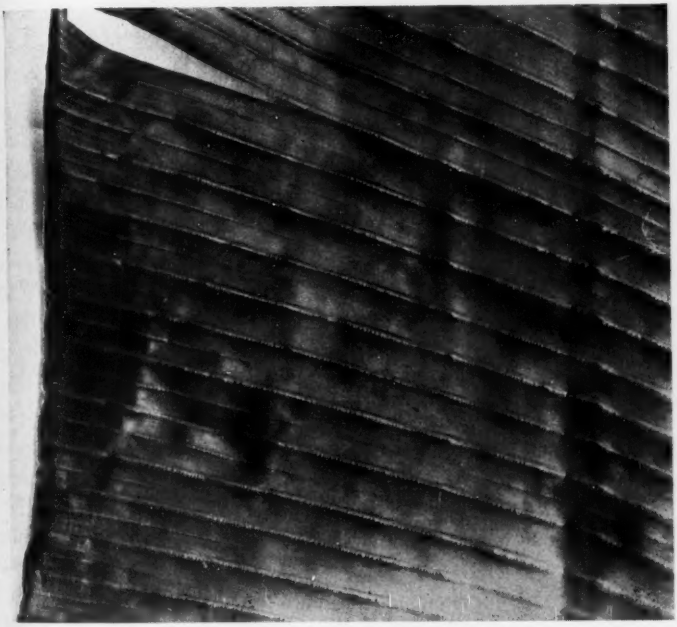


FIG. 12.—Upper surface of left side of lamina bound 12 days (note swellings above veins and backwardly rolled margin).

tween the principal veins, or to elevate the latter. The ribs formed on these bound leaves are always much stronger on the left or outer side of the lamina than on the right, and this difference persists even if the leaf is allowed to remain bound for a long period (three weeks or more). Near the right margin of a bound leaf the lamina never becomes strongly ribbed, and is in marked contrast to the opposite side. On each half of the lamina the ribbing is stronger next to the midrib than at the margin.

The thickness of the upper water tissue varies according to the

region of the lamina. Near the margins it is often single layered but usually double, near the midrib it becomes triple, and still closer to it, it is often quadruple. Immediately adjacent to a principal vein there is often one more layer of water tissue than elsewhere in the same region. The extra layer may extend as far as the fifth or sixth veinlet on either side of the principal vein. As seen in surface view, the cells of the water tissue are elongated transversely to the veins. The cell sap is perfectly clear, as in typical expansion cells, without chlorophyll or highly refractive bodies. In the bound leaves, at places where the water tissue consisted of two or three layers, the outermost layer was commonly much less hypertrophied than the one or two lying beneath it, and sometimes the cells comprising it were hardly deeper than normal. As a result of binding, the thickness of the water tissue above the principal veins may exceed that of all the remainder of the cross-section of the leaf. Thus at one point the water tissue became 480 μ deep, while the entire thickness of the lamina at this place was only 816 μ. The longest cell of the water tissue at this point measured 256 μ in depth, and cells 320 μ in depth have been observed. Cells of the epidermis do not aid in unrolling, and when the leaf is bound, do not exhibit any noteworthy hypertrophy, but are passively raised by the elongating cells of the water tissue. In some places swelling of the tissue above the principal veins was so great that the epidermis was split and a fissure extended into the cushion of tissue. The lumina of the fibers lying above the principal veins are strongly extended in the anticlinal direction, so that the fibrous bundle projects far upward into the cushion of water tissue. The palisade cells immediately adjacent to the principal bundle are also elongated somewhat more than normal. The tissues on the lower side of the leaf are not affected by binding it so that it cannot unroll.

On the plantation and in the jungle, one occasionally finds leaves of the Scitamineae with ribs abnormally swollen because the emerging lamina was caught in the embrace of a twining vine and unable to expand. If the plant is sickly, or if for any reason the leaf does not emerge normally and the lamina cannot unfurl at its due period, hypertrophy of the water tissue usually results. Local hypertrophy occurs wherever a sharp fold has been formed in vernation.

Prevention of the normal unrolling of the leaf also causes great hypertrophy of the water tissue on the upper side of the pulvinar bands (fig. 10). Here too the cells of the outermost layer are hardly swollen beyond the normal. The turgor of this tissue causes the lamina halves to become strongly deflexed beneath the midrib when unbound, even in moist weather and in the morning, when the other leaves are spread out horizontally; but after several days, by a process described later, they are raised to the normal position.

Unrolling of lamina in darkness

When a leaf has been constrained in the coiled condition by binding, even after a long period the right side remains yellowish, especially at the margin. The slight production of chlorophyll on this side seemed to be correlated with the very slight ribbing which was acquired, and suggested that light was essential to the unrolling of the leaf. To test this supposition, young suckers in the field were inclosed in light-proof portable houses, 90 cm. square by 2 m. high, which were equipped with light-proof ventilators at the top and bottom, and draped with white sheets to prevent their becoming too hot in the sun. The leaves which emerged in these houses unrolled normally, although they were without a trace of chlorophyll.

As in the leaves in the open, the formation of ribs began at the apex on the left side, and proceeded thence basally and to the right. The order of maturation of the expansion cells is thus an autonomic process, adjusted to bring about the harmonious unrolling of the leaf, and is not, as at first suspected, determined by the incidence of light. Their behavior in the banana is in entire agreement with the results of Löv's studies of a number of other plants. In leaves folded into a cylinder, with their edges in contact, such as those of *Luzula nemorosa*, the order in which the cells of the upper epidermis enlarge is from both margins toward the center. In simply folded leaves, such as those of *Hemerocallis*, enlargement of the expansion cells also proceeds inward from the margins. In convolute leaves the order of enlargement is from the exterior margin toward the interior. In involute leaves, as exemplified by species of the Commelinaceae, the order of enlargement is from the midrib toward both margins, while in the palms the expansion cells of the marginal folds are the first to enlarge.

Although it thus became evident that the beginning of the enlargement of the cells of the upper water tissue at the left margin was an autonomic process, not conditioned by the greater exposure of this portion of the leaf to the light, it remained to be seen whether light influences the degree of hypertrophy which can be attained by these cells. For this purpose leaves were bound as they emerged in the dark-house. After remaining bound for 16 days from the time it had completely emerged, a leaf was unbound and revealed a slight to moderate degree of hypertrophy of the cells of the upper water tissue in the neighborhood of the principal veins on the left side; but enlargement of the cells was not nearly so great as one would find in a leaf held bound for a similar period in the light. The anticlinal length of the longest cell observed was 130 μ, and the 3-layered water tissue at this point was 176 μ thick, while the total thickness of the lamina was 416 μ. The degree of enlargement and the turgor attained by the expansion cells are increased by light, although an enlargement normally sufficient to unroll the lamina is independent of light.

When the experiment was terminated, and the dark-houses, which were equipped with handles and could be lifted by four men, were removed from over the plants, the leaves turned green in inverse order of their age; the youngest in about 24 hours. In the leaves which had been longest in darkness, chlorophyll was produced first along the principal and stronger subordinate veins; only later in the intermediate regions. At the apex of the first leaf which had emerged in the dark-house, these veins alone turned green. These observations seem to indicate that which is confirmed by microscopical examination, that the tissues along the upper side of the principal veins are the last to lose their embryonic character, since their peculiar function demands their maturation at a later period than the rest of the leaf.

Effect of premature unrolling

The preceding experiment suggested that the compression to which these cells are normally subjected, by their position on the concave surface of the rolled leaf, is responsible in the first place for their normal enlargement, and hence for the production of their ribs. A lamina which was just appearing above the false stem of a young sucker, and which had not yet become ribbed, was freed

by cutting away all the leaf sheaths covering it. The left side was carefully unrolled, then rerolled around the midrib and right side as tightly as possible, but with the upper instead of the lower surface facing outward. It was then bound by cord in this position and supported to relieve the strain on the still immature petiole. The left side of leaves treated in this manner never became ribbed, but the upper surface remained smooth as in immature leaves, even after being bound for 3 weeks. The right side, which was allowed to remain rolled in the normal direction, became strongly ribbed, as in the bound leaves already described.

Rerolling the leaves with the ventral surface convex places the cells of the upper water tissue in an unnatural position, and it was desirable to determine the effect of merely flattening out the lamina. This experiment was attended by considerable practical difficulty. The framework which supports the lamina must hold it perfectly flat, and yet permit it to slip upward as the sheath of the leaf elongates. The leaf used must be of exactly the right age, for if too young it will be unable to withstand the exposure to which it is subjected by premature unrolling, and if too old, will already have begun to become ribbed. Finally these requirements were met by constructing a framework consisting of two parallel series of bars of split bamboo. A lamina about 1 m. long, which had emerged for one-quarter to one-half its length, from a young sucker, was chosen. It was freed of the surrounding leaf sheaths, the left side unrolled, the framework slipped over it and set into the ground beside the plant. During the first few days, or until the tissues had become green, it was shaded by tying cut banana leaves to the outside of the frame. The left side of these leaves became at most very slightly ribbed, always much less than in normal leaves. In small restricted areas, which had not been held perfectly flat in the frame, the ribs were much stronger, and in some places the expansion cells were even somewhat hypertrophied, showing that a slight concavity of the upper surface is sufficient to cause ribbing.

Leaves which were prematurely unrolled and did not become normally ribbed, usually hung more or less limp from their midribs instead of being spread out horizontally in the morning and evening, and on wet days. The ribs are necessary for the mechanical support

of the broad flat lamina, and the expansion cells, in addition to the function of unrolling the lamina, must create the framework for its support.

Effect of rerolling mature leaves

Two mature leaves, the youngest completely unfolded leaf, and the one next below this on a young sucker, were loosely rolled into the position of vernation and held by binding with cord. After 9 days they were unrolled and examined. The younger leaf had been affected by this treatment much more than the older. The water tissue above and beside the principal veins of the former was strongly hypertrophied, and the leaf much more conspicuously ribbed than normal. When the water tissue was three layers thick only the inner two layers were hypertrophied; when two layers thick, only the inner. The hyaline cells on the upper side of the pulvinar bands, particularly on the right side, which was the more strongly inrolled, were also considerably hypertrophied. The older leaf showed the same symptoms in a reduced degree.

Development of pulvinar band

At night, in the early morning and late afternoon, and continuously on wet days the two sides of the lamina stand out in a plane on either side of the midrib. In the middle of bright sunny days they bend beneath the midrib, and eventually, if the day is dry the dorsal surfaces of the two sides are brought into contact. Since the stomata are situated principally on the lower side of the lamina, and are thereby brought into a more protected position, and moreover because by the assumption of the profile position the absorption of sunlight is greatly decreased, the water loss must be considerably diminished, although at present no actual measurements on this point are available. When a leaf has been torn into strips by the wind, the individual strips behave as though the lamina were still intact. If the sky suddenly becomes overclouded or there is an unexpected shower during the middle of a day which was at first sunny, recovery of the horizontal position by the lamina halves is rapid.

That the halves of the lamina do not passively sink down as the result of wilting may easily be demonstrated by holding such a leaf in an inverted position, when the lamina halves will not droop down

but remain folded upward. Their movements are determined by changes in the turgor of the pulvinar bands which border the midrib on each side, and the flexure is sharply localized in these bands. It therefore becomes of interest to examine their structure. Their width is about 5 mm. in large leaves, and in thickness they taper from about 1.8 mm. next the midrib to 1.2 next the lamina half. Fig. 7 represents a portion of the cross-section of a pulvinar band of a leaf of medium size, which was just appearing from the false stem. It shows the condition found in the mature leaf tolerably well, except in the form of the cells lying immediately below the upper and above the lower epidermis. The change which occurs in the cells of the upper water tissue during the process of expansion has already been described. The central portion of the organ is characterized by the absence of lacunae (although small spaces between the rounded cells are numerous), the many vascular bundles, and the conspicuous sheaths of fibers which accompany them. Toward the lower surface bast bundles are numerous, surrounded by exceedingly thick sheaths of fibers, and still lower in the cross-section strands of fibers alone are found. Above the lower epidermis two layers of collenchymatously thickened cells occur. It is the behavior of these cells, and those immediately above them, which is of particular interest.

When the leaf first expands, the great turgor engendered in the upper water tissue of the pulvinar bands causes the halves of the lamina to become reflexed below the midrib (fig. 1). After a few days the lamina becomes flattened out again. This is brought about by the gradual enlargement of those cells on the lower side of the pulvinar band which lie between the hypodermal layer and the lowest of the bast bundles. Their elongation does not begin until the leaf has expanded, and is completed in about 10–12 days. The process of elongation was studied by removing with a sharp scalpel little rectangular pieces from the apex, middle, and base of each pulvinar band of a single leaf at intervals of 4 days. The leaf was so large that the loss of these small pieces of tissue did not seem to affect it, and successive samples in each region were spaced a few centimeters apart, so as to avoid wound reactions. The thickness of the tissue formed by these elongating cells, and the length (anticlinal) of the longest cell in each region, were measured by a micrometer scale,

and the results of one series of measurements are recorded in table I. In the mature leaf the tissue is composed of enormously elongated, irregularly prismatic cells, with clear contents and containing few chloroplasts (fig. 8).

Very young leaves do not respond to changes of atmospheric conditions by rising and sinking. The diurnal movements do not become

TABLE I

DEVELOPMENT OF PRISMATIC CELLS OF PULVINAR BAND; TOTAL THICKNESS OF PRISMATIC TISSUE IN MICRONS

POSITION	March 1*	March 5	March 9	March 13	March 25
Left side					
Apex	64	224	272	336	384
Middle	96	304	400	544	512
Base	96	320	416	528	496
Right side					
Apex	80	272	304	400	448
Middle	96	368	400	512	512
Base	96	384	400	400	496
	Length of longest cell in three sections				
Left side					
Apex	26	122	130	204	204
Middle	26	115	207	229	233
Base	30	144	267	241	215
Right side					
Apex	30	155	163	222	233
Middle	26	144	192	241	218
Base	26	144	174	167	207

* On March 1 the base of the lamina had just become free from the false stem; the cells on the lower side of the pulvinar band were not in the least elongated, but appeared as in fig. 7.

pronounced until after development of the prismatic cells. The movements seem to be the result of variations in turgor of the two antagonistic layers, the upper water tissue and the lower prismatic tissue. In a segment of a cut leaf the latter loses water more rapidly, and the lamina halves bend downward beside the midrib. Placed in water, the prismatic cells rapidly regain their turgor, and the lamina halves are again spread in a plane.

The conditions necessary for development of the prismatic cells are pressure and light. The pressure is normally supplied by the antagonistic action of the upper water tissue, which, by its strong

development when the leaf first expands, bends the pulvinar band downward and throws the cells on its lower side into a state of compression. In the experiments in which unrolling of the leaf was prevented by binding, the cells on the lower side of the pulvinar band did not become prismatic. When the left side of the lamina was unrolled and then rerolled in the reverse direction, development of prismatic cells on this side was very irregular, because in bending the leaf back the flexure generally occurred in the lamina itself rather than in the thicker pulvinar band, and the lower surface of the latter only occasionally became concave. On the right side in these experiments no prismatic cells were formed. When the time of unrolling of the lamina was delayed a week or two by binding, and the water tissue on the upper side of the pulvinar band was greatly hypertrophied, the lamina halves became strongly reflexed after the cord was removed, but gradually bent upward as the prismatic cells developed. Leaves which expanded in darkness never developed prismatic cells so long as they were left in the dark-house. Since, as a result of the slighter enlargement and turgidity of the cells of the upper water tissue of the pulvinar band, the leaves which unfold in darkness never become so strongly reflexed along the midrib as leaves which emerge in the light, there was a possibility that failure of development of the prismatic cells was a result of insufficient compression of the lower side of the pulvinar band. Leaves were bound as they emerged in the darkness and the unfurling delayed a week or more, which resulted in a slight hypertrophy of the upper water tissue, and when unbound the lamina halves became more strongly reflexed. Even under these circumstances there was no development of prismatic cells in darkness. The appropriate cells retain their capacity to elongate, however, and even after their development has been delayed 20 days by darkness, when returned to the light they enlarge and become prismatic.

In all of the species which I have been able to examine, including *Musa textilis*, *M. tomentosa*, *M. malaccensis*, *M. sanguinea*, *M. rosacea*, and many varieties of *M. sapientum*, the pulvinar band with its prismatic cells is well developed, and the lamina halves fold downward as the leaf loses water. Although there is great development of fibers, undoubtedly made necessary by the strategical position of the

organ, in the pulvinar bands of all of these species they are in general weakly or not at all lignified. The same strand of fibers is usually strongly lignified on both sides of the pulvinar band, that is, in the midrib and the lamina half, but in the band itself it is either not lignified or its lignin reaction is much weaker than in the adjacent regions. The failure of lignification is typical of pulvini, and is of advantage to an organ which must bend repeatedly. I have already described (5) a peculiarity of the cuticle over the pulvinar band of *M. sapientum* which seems associated with the motility of this organ.

The genus *Musa* is the only member of the Scitamineae known to the writer the leaves of which fold downward in dry weather. In all others (including the species described later) the leaves fold upward, exposing the lower surface, in which the stomata are principally situated. In these species there are no distinct pulvinar bands, and the bundles of fibers are continuously lignified as they pass from the midrib to the lamina halves. Like the majority of monocotyledonous leaves, these reverse the movements of unfolding as they dry (3). GOEBEL (2) points out that the movements of plant organs must be considered primarily in relation to their development, external and internal symmetry, and mode of unfolding. An organ performs movements because its structure is asymmetrical (whether or not the lack of symmetry is externally evident), and opposite sides are differently affected by various external or internal changes. The movement may be performed first as the organ expands, and subsequent movements of the mature organ may be merely reversals and repetitions of the act of unfolding. Since the movements of plants are determined by structural peculiarities which frequently originate in response to needs or conditions unrelated to these movements, they may or may not happen to prove useful, but are not of necessity useful. This certainly is true of the movements of many monocotyledonous leaves in dry weather. The leaf tends to return to the position it occupied in vernation, because the expansion cells, which by their turgor spread out the lamina halves and hold them so, lose their turgor and permit the antagonistic forces of the tissues on the opposite side of the leaf to reverse the process of unfolding. Far from being of advantage to the plant, these movements appear just the reverse, for the stomata are thereby more exposed. But because of

development of the prismatic tissue of the pulvinar bands, movements of the lamina in dry weather are not mere reversals of the act of expansion, and appear to be advantageous to the plant. Like the sleep movements of the leaves of *Tropaeolum majus*, which bend outward at night although the upper surface is directed inward in the immature leaf, they are exactly opposite to the movements of unfolding. The prismatic cells of the pulvinar band appear to be a late acquisition in the phylogeny of *Musa*, since its nearest relatives, *Ravenala* and *Heliconia*, lack them; they are certainly a late development in the ontogeny, and originate only under special conditions of pressure and light.

Unfolding of leaves in other genera of Scitamineae

Musaceae

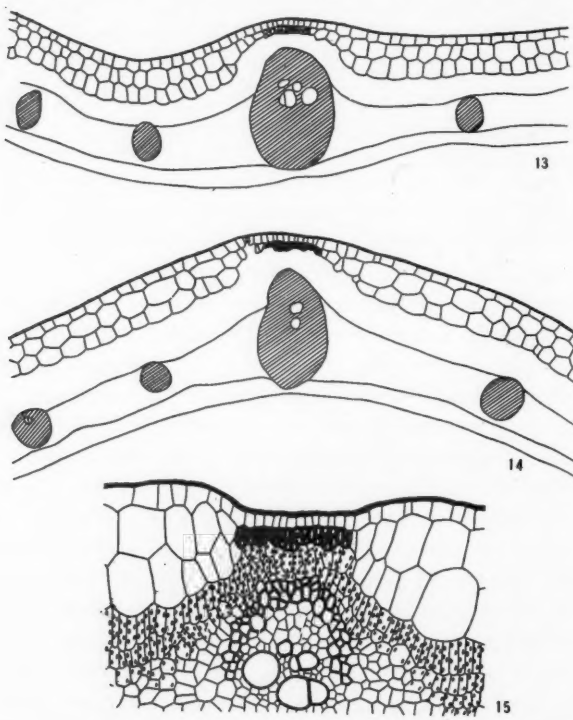
Heliconia bihai L.—The long, slender leaves of this plant emerge very gradually from the short false stem, and the apex has usually spread out long before the base has escaped from between the sheaths of the older leaves. The upper water tissue is everywhere (except close to the midrib) but a single layer in thickness. Unrolling of the lamina halves and formation of transverse ribs proceed almost exactly as in *Musa*. The prevention of unrolling by binding with cord caused the water tissue above and immediately adjacent to the principal veins to become enormously swollen, forming thick welts running across the lamina half. Single cells reached 432 μ in anticlinal length, about 3–4 times the normal depth. The thickness of the hypertrophied water tissue often exceeded that of the remainder of the leaf, and at one place, where the total cross-section was 560 μ, it occupied 352 μ. The water tissue above many of the stronger subordinate veins was hypertrophied, and in places where the upper surface was particularly concave (the lamina does not form a perfect helix in vernation) the hypertrophy extended completely across the interval between the principal veins.

In dry weather the leaf folds upward, exposing the stomata, as do those of all other species of *Heliconia* with which I am familiar.

Zingiberaceae

Alpinia exaltata (L.) R. & S.—The aerial shoots of this species, attaining 5 m. in height, consist of a slender stem everywhere sur-

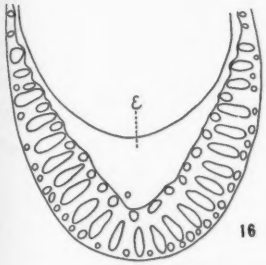
rounded by many thicknesses of the long overlapping sheaths, and are continued upward into a false stem composed of these sheaths alone. When a new lamina emerges it is practically full-grown. If such a leaf is unrolled, it is found to be perfectly flat and without

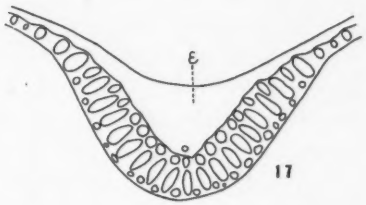


FIGS. 13–15.—*Alpinia exaltata:* Fig. 13, cross-section through principal vein of leaf just beginning to emerge; only water tissue and epidermis shown in detail; camera lucida ×50; fig. 14, same, from fully expanded leaf; ×50; fig. 15, cross-section through upper portion of principal vein of leaf bound 14 days; ×112.

ribs, but it feels slightly rough if the finger is drawn across it transversely to the veins, which project slightly above the surface (fig. 13). Even the strongest veins do not occupy the entire interval between the upper and lower water tissues, as in *Musa*, but the palisade tissue is continuous across them. The two-layered upper water tissue

is interrupted above the stronger veins by a group of small cells, the walls of which become thickened even before the leaf appears at the top of the false stem. For this reason the process of unrolling differs from that in *Musa* and *Heliconia*. The cells of the water tissue on either side of the principal veins are the chief expansion cells, and by their enlargement cause the lamina to become strongly ribbed (fig. 14). Prevention of unrolling by binding does not cause the enormous hypertrophy characteristic of all the other Scitamineae studied, probably because the water cells are interrupted by cells which are thick walled and incapable of elongating, and hence hold the others





FIGS. 16, 17.—*Alpinia exaltata:* fig. 16, cross-section through midrib of leaf before expansion; fig. 17, same, from expanded leaf; *e*, expansion cells; ×5.

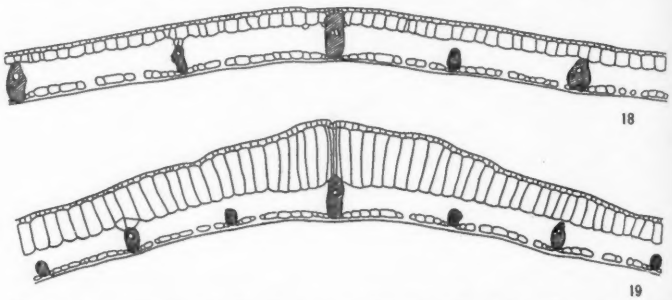
back. There is, however, a definite though small hypertrophy of the upper water tissue extending completely across the interval between the principal veins. Fig. 15 shows the region above a principal vein of a leaf which had been bound for 2 weeks. The water tissue bulges up above the mechanical tissue on either side, but appears to be held in check by the attachment of the epidermis to the latter. The outer side of the bound lamina exhibits other symptoms of the high turgidity of its expansion cells, such as splitting of the tissue and rolling backward of the margin, and feels smoother and more turgid than the normal leaf.

Fig. 16 shows how the clear transparent expansion cells occupy the entire central portion of the cross-section of the midrib, while the vascular bundles are all crowded to the lower side. At the proper time the former increase in size and turgidity, spread the midrib, and flatten out the lamina halves (fig. 17). On drying, the lamina halves

fold together again, as a result of the loss of turgidity by these strategically situated cells. The stomata, situated chiefly in the lower surface of the leaf, are thereby exposed.

CANNACEAE

Canna sp.—The *Canna* chosen for study is a species with small red flowers commonly cultivated about the province of Bocas del Toro, Panama, and often found growing spontaneously. It is apparently undescribed, and in the unsatisfactory state of the taxonomy of the genus it appears undesirable to make a new species of it without a revision of the group. The leaves of *Canna* species show great uni-



FIGS. 18, 19.—*Canna* sp.: fig. 18, cross-section through principal vein of normally expanded leaf, showing water tissue; fig. 19, same, from leaf bound 7 days; ×25.

formity in structure, however, so specific identity is not important for the present purpose.

Canna is the only genus of the Scitamineae studied by Löv, or about which there is any published account of the mode of unrolling of the lamina. Löv recognized that the water tissue furnishes the principal expansion cells, but failed to notice the greater enlargement of those above and adjacent to the principal veins, which results in the formation of the ribs so essential to the mechanical support of the lamina throughout the order. The epidermis plays an inconsiderable part in the act of unrolling. Löv also gives some interesting observations on the substitution of other tissues in the act of unrolling when the water tissue is destroyed. By scratching the upper surface with a sharp knife, it is possible to injure the water tissue without destroying the epidermis. In this case the latter cells become

much deeper than normal, and the leaf continues to unfurl. When the epidermis and water tissue are both destroyed by touching with a glowing needle, an abnormal growth of the palisade cells takes place, particularly in the neighborhood of the large veins, where periclinal divisions of the cells occur. The leaf manages to unroll.

The leaf which is just beginning to emerge is unribbed. Formation of the weak ribs is accomplished without the cells of the single layered water tissue becoming much larger above and adjacent to the principal veins than midway between them (fig. 18). Prevention of unrolling by tying caused the great hypertrophy of the cells of the upper water tissue (fig. 19). It was most pronounced above the principal veins, but extended completely across the leaf, with lesser maxima above many of the stronger subordinate veins. The lamina became strongly ribbed. The hypertrophied cells of the water tissue measured as much as 384 μ in depth, which is 4–5 times the normal, and accounted for over half the cross-section of the leaf. The epidermal cells were also greatly enlarged, becoming in places 67 μ in depth, as opposed to 15–26 μ in the normally unfurled leaf. The leaves curled backward when unbound and placed in water.

The leaves of *Canna*, like those of *Musa*, unroll normally in darkness.

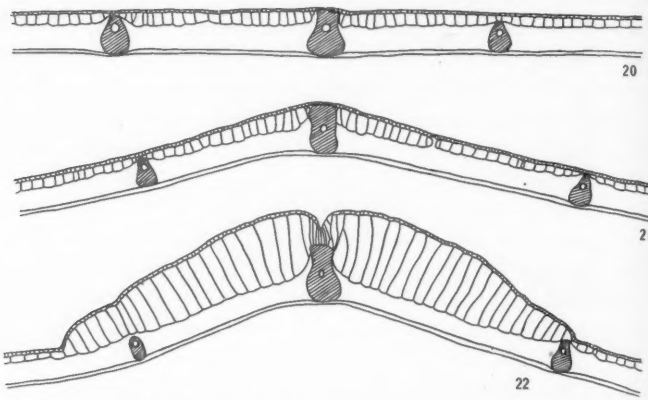
Marantaceae

Calathea magnifica Morton & Skutch.[4]—The leaves of this newly described species are ovate-oblong and very large, reaching 114 cm. long by 58 cm. broad. At the time the lamina appears above the short false stem, and even after the apex has begun to turn green, the upper surface is perfectly smooth, without the ribs so conspicuous in the mature leaf (fig. 20). The fibers on the upper side of the vascular bundles are thin walled, while those on the lower side are already conspicuously thickened. The water tissue is single layered everywhere except in the immediate vicinity of the midrib. It is usually not continuous above the principal veins; the fibers on their upper side are either in direct contact with the epidermis or separated from it by a few small scattered cells which appear to belong to the water tissue. In unfurling, the cells on either side of the principal

[4] Jour. Wash. Acad. Sci. **20**: 372. 1930.

veins enlarge considerably, with the result that the leaf becomes ribbed (fig. 21).

Prevention of unrolling by binding the leaf causes great hypertrophy of the cells of the water tissue in the neighborhood of the principal veins. The region of hypertrophy is laterally sharply delimited from the surrounding water tissue (fig. 22). Single cells 464 μ in depth were observed, 2.5–3.7 times the depth found in normal leaves. Where it was thickest, the water tissue accounted for two-



FIGS. 20–22.—*Calathea magnifica:* fig. 20, cross-section through principal vein of leaf before expansion, showing water tissue; fig. 21, same, through normally expanded leaf; fig. 22, same, through leaf bound 8 days; ×23.

thirds to three-fourths the entire cross-section of the lamina. There was generally a deep furrow in the cushion of tissue above the center of the vein, except in the rare cases where the water tissue was continuous above it. This occurred because the epidermal cells, here in contact with the fibers, were unable to elongate as rapidly as the cells of the water tissue, which for this reason bulged above them. The epidermal cells in this position did undergo an enormous elongation, becoming 44–78 μ in depth when normally they were 7–11 μ, but they appeared to have been passively stretched by the water tissue (fig. 22).

As in the other species described, not only must the two halves of the lamina unroll individually, but it is necessary that they bend

outward along the sides of the midrib. This is accomplished by the great development of prismatic expansion cells, with clear cell contents, in these regions. The largest vascular bundles are situated near the upper side of the organ, and numerous smaller ones are scattered beneath (fig. 23). The expansion cells penetrate deeply between the largest bundles, and by their enlargement at the time of unfurling of the leaf bend back the lamina halves. In drying they lose their turgor, and the lamina halves bend upward. In the middle of sunny days it is striking to see the large leaves of these plants, which grow in dense stands in low open places, all folded together upward, exposing the white, wax encrusted lower surface, in which most of the stomata are situated.

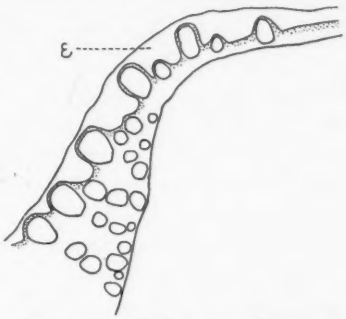


FIG. 23.—*Calathea magnifica:* cross-section through junction of midrib and lamina half; *e*, expansion cells; ×12.

Summary

1. An experimental study was made of the mechanics of the unrolling of the leaves of *Musa* and *Heliconia* (Musaceae), *Alpinia* (Zingiberaceae), *Canna* (Cannaceae), and *Calathea* (Marantaceae), representing each of the four families of the tropical order Scitamineae.

2. The large laminae of all the species studied are convolute in vernation. They are practically full-grown at the time they begin to emerge from the top of the false stem, which, to a greater or less degree, is characteristic of most of the genera of this order. At this time, if the lamina is unrolled, it is found to be smooth and flat, without the transverse ribs so characteristic of these plants.

3. Unrolling is effected by the timely enlargement of the cells of the upper water tissue above and immediately adjacent to the principal veins. The localized enlargement of these cells results in raising the principal veins above the general surface of the lamina, thus forming the ribs. In the coiled position of the leaf they are pushed inward, come to occupy a curvature of smaller radius, and are thus

(with the turgid cells which lie above them) brought into a state of compression. In this compressed state they force the lamina to unroll.

4. In *Musa* the fibers on the dorsal side of the veins are thick walled and become lignified somewhat before the portion of the leaf in which they lie begins to unroll. The fibers on the ventral side remain thin walled until the leaf has unfolded, and never become lignified.

5. Enlargement of the expansion cells proceeds inward from the outer margin of the lamina, and from the apex toward the base.

6. If the unrolling of the leaf is prevented by binding it with cord, the expansion cells in all species studied except *Alpinia* become greatly enlarged, and occupy over half the entire cross-section of the lamina. In *Alpinia* the hypertrophy is relatively slight, apparently because the water tissue is interrupted by thick walled cells which are not able to stretch. Only in *Canna* does the hypertrophy extend completely across the lamina half; in most cases it is strictly limited to the region of the principal and the stronger subordinate veins.

7. Leaves of *Musa* and *Canna* were found to unroll in complete absence of light. In darkness, however, the hypertrophy of the water cells of bound leaves is much less than of those in the light.

8. If the lamina of *Musa* is prematurely unrolled and held in a plane, the expansion cells fail to enlarge. They enlarge only when in a state of compression.

9. The ribs are essential to impart rigidity to the lamina halves, which are otherwise without lateral support.

10. In addition to the unrolling of each side of the lamina, a special arrangement is necessary to bend it outward from the midrib. This is effected by great development of expansion cells along the edges of the midrib (*Musa*, *Heliconia*, *Calathea*), or in the center of the midrib (*Alpinia*).

11. In most members of the Scitamineae, when the leaf suffers great water loss and the expansion cells lose their turgidity, the process of unfolding is in part reversed. Each side of the lamina bends upward along the midrib, exposing the stomata, which are situated principally in the lower surface. This non-adaptive movement is a necessary result of the structure and method of unfolding of the leaf.

12. In *Musa* the sides of the lamina fold downward in dry weather. This is a result of the formation of a rather massive tissue of prismatic cells in a strip along either side of the midrib, which thus becomes a pulvinar band. The rising and falling of the lamina halves are brought about by differential changes in the turgor of two antagonistic tissues, the expansion cells on the upper side and the prismatic cells on the lower.

13. The prismatic cells begin to develop only after complete expansion of the leaf, when stimulated by the compression to which they are subjected after the expansion cells on the upper side of the pulvinar band bend it downward. Hence they do not develop in leaves which are prevented from unfolding by binding. They develop only in the light.

In conclusion, I wish to express gratitude to Professor DUNCAN S. JOHNSON for his continued interest in the course of my tropical studies; to Dr. JOHN R. JOHNSTON, Director of Agricultural Research of the United Fruit Company of Boston, for allowing me the use of the Company's research stations in Central America, and for many other courtesies extended to me by the Company; and to Mr. JOSEPH H. PERMAR, Director of the station at Almirante, Panama, where the present study was completed, for his unfailing cooperation in my work.

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POLLEN TUBE GROWTH AND CONTROL OF GAMETOPHYTIC SELECTION IN COCKLEBUR, A 25-CHROMOSOME DATURA[1]

JOHN T. BUCHHOLZ AND ALBERT F. BLAKESLEE

(WITH THIRTEEN FIGURES)

Introduction

Among the primary ($2n+1$) types of *Datura stramonium*, several have been found which may be transmitted through the pollen in back-crosses to normal $2n$ plants. We have selected Cocklebur (Ck) as one for special study, together with Wedge (Wd), its secondary.

POLLEN TUBE GROWTH FOR COCKLEBUR POLLEN

An analysis by means of pollen tube growth tests of the pollen from Ck reveals distinctly the presence of two classes of pollen tubes. Figs. 1–4 are made from counts of the ends of pollen tubes in the style after 12 hours' growth; figs. 7 and 8 are similar tests after 16 hours' growth. The normal appearing pollen tubes are plotted above the datum line in the appropriate 2 mm. interval[2] corresponding to their distance from the stigma, while the abnormal appearing pollen tubes are plotted below the datum line. The shaded vertical bar at the left in each diagram indicates the number of pollen grains remaining ungerminated on the stigma. Aborted pollen grains could be observed but could not be counted reliably on the microscopic preparations and are not included, but their proportion has been determined by CARTLEDGE (4).

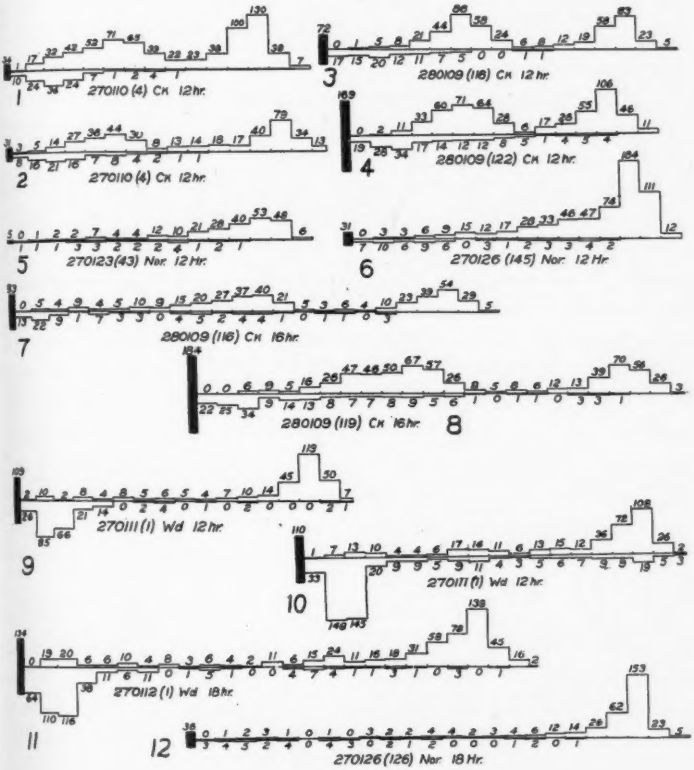
The figures of the Ck tests all show a distinct bimodal grouping; two groups of tubes of nearly equal proportions, when we allow for the usual proportion of overlapping of stragglers from the longest group which may be included in the second group. The inference from an examination of these distributions is that these are the two

[1] This cooperative investigation was made possible by grants from the Joseph Henry Fund of the National Academy of Sciences.

[2] In some previous publications we have used 3 mm. intervals for plotting, and in others (10), including this paper, we are using 2 mm. intervals.

classes of pollen tubes, with $(n+1)$ and n chromosomes respectively.

Figs. 5 and 6 represent similar tests made as controls, in which the pollen of $2n$ plants was used. Here we have pollen tubes with n



FIGS. 1–12

chromosomes which are grouped chiefly in a single mode of distribution at a distance which corresponds to the position of the longest groups in figs. 1–4. The inference is, therefore, that the n pollen tubes in figs. 1–4 make up the first or longest group, while the $(n+1)$ pollen tubes make up the second group. This distribution indicates that either the $(n+1)$ pollen tubes germinate after several

hours' delay, or they grow more slowly. We have determined these points somewhat roughly and found, by comparing and plotting these two modes in a series of tests after various successive time intervals, that both the period of germination is prolonged and the rate of growth of these $(n+1)$ pollen tubes is slower than for those with n chromosomes. At a temperature at which the growth rate of the first mode was 2.6 mm. per hour, that of the second mode was 1.9 mm. per hour. The delay in germination and slower growth seem to place the group in the second mode at less than half the distance reached by the first, in figs. 1–4.

In figs. 1 and 2, from tests made in 1928, only 5–6 per cent of the pollen grains remained ungerminated on the stigma, but in figs. 3 and 4 of the following summer 11.5 and 19.5 per cent respectively remained ungerminated. The relative proportion of pollen tubes in the two groups is not greatly modified, however, nor is the proportion of burst pollen tubes altered consistently in these or the 16-hour tests of figs. 7 and 8. Figs. 1 and 2 show tests made under very favorable conditions of pollen development, when the styles used for testing as well as the pollen parents seemed to be at their best, while figs. 3, 4, 7, and 8 were made when these conditions were somewhat less favorable. It is obvious that under the conditions of either of these tests the $(n+1)$ pollen tubes may reach the ovary in considerable proportions, since the style usually remains attached for two or more days after pollination.

If a great amount of pollen is applied to the stigma in making pollinations, the slow growing $(n+1)$ tubes will have only a small chance of accomplishing fertilization, because the pollen tubes with n chromosomes may fertilize all of the eggs before the arrival of the $(n+1)$ group. In making routine pollinations the pollen is usually crowded. If only a medium or a small amount of pollen (400-500 grains) is used in making the pollinations, however, there is some chance for fertilization by $(n+1)$ pollen tubes, since the ovary of a well developed flower may have 400–600 ovules. The question has also been tested with the $(2n+1)$ Globe, in male back-crosses using counted pollinations, when the optimum number of pollen grains for the transmission of the extra chromosome was found to be around 500 (**14, 15**).

We have frequently found evidence of pollen transmissibility of Ck in connection with routine breeding experiments. Some of these are given in table I. Not only is Ck transmitted through the pollen from Ck plants, but its secondary Wd transmits the Ck through the pollen, while the Wd itself is generally not transmitted. This relationship between Ck and Wd will be considered further in a later paragraph.

Special pollination methods and excision of styles

Late in the summer of 1927 it was decided to put to a more critical test the question of pollen transmissibility in Ck, in the hope of de-

TABLE I

Pollen transmissibility of Cocklebur and Wedge in garden plants from ordinary crosses with four lines

	Cocklebur						Wedge						
	Ped. no.	Plants recorded	Normal	Ck	Per cent	Wd	Ped. no.	Plants recorded	Normal	Ck	Per cent	Wd	Per cent
Line 21....	230231	181	171	10	5.5	0	230232	180	170	8	4.4	2	1.1
Line 26....	230250	174	166	8	4.6	0	230251	180	180	0	0	0	0
Line 33....	230268	151	128	23	15.2	0	230269	199	191	8	4.2	0	0
Line 36....	230291	102	86	16	15.7	0	230292	105	103	2	1.9	0	0
Totals.....		608	551	57	9.2	0	Totals...	664	644	18	2.7	2	0.3

veloping experimental methods by which we might increase or decrease this transmissibility. Pollen tube growth tests made late in August showed good pollen germination and a striking bimodal grouping of the pollen tubes, indicating favorable conditions of pollen at the time of making these tests. In order to control pollen tube growth conditions as far as possible, potted plants from line 1A were used and kept at a uniform, moderately low temperature in the basement of the laboratory during the period of pollen tube growth. Favorable pollen parents were used and the condition of temperature was similar to that during the earlier tests, including those represented in figs. 1 and 2.

The methods chosen for control of the competition between pollen tubes were those used in our studies of pollen transmissibility of $(2n+1)$ Globes. One of these consists of restricting the pollinations to a small or medium number of pollen grains (15). With this meth-

od we combined style-excision operations, the styles of some of the flowers being cut off after the longest pollen tubes had entered the ovary, in order to exclude the second slow growing group. The pollen grains were not counted, but the numbers were roughly estimated as the pollinations were made, by using small camel's-hair brushes and examining these under a Greenough binocular microscope in order to estimate the quantity.

While the *2n* plants used as females were potted plants, the pollen used was taken from Ck plants growing in the garden whose pollen had been showing at the time a high percentage of germination and favorable conditions of pollen tube growth.

Three parallel sets of pollinations were made, beginning on an afternoon, and all plants pollinated were kept under similar conditions. Flowers of set A were used for examination of pollen tube growth in the style from time to time on the morning of the following day, in order to determine the progress of the longest group of pollen tubes and to predict the time of their arrival at the ovary in the remaining plants. Those of set B were left undisturbed, and those of set C were used for style-excision experiments. The styles of set C were cut off when the longest group of pollen tubes had entered the ovary, as predicted by examination of rapidly prepared tests of set A. This operation excluded the second or slow growing group illustrated in figs. 1 and 2. Actually it was found that we had excised most of the styles a little too soon; and while the parallel set A indicated that the ends of the pollen tubes had actually entered the ovary (which was also borne out by a later experiment of the styles cut from set C), it appeared that many of the pollen tubes were so shocked or injured in this operation that they failed to continue growth; thus the seed yield was lower than desired.[3]

While the seed yields from the style-excision experiments were low, it is obvious that we were successful in admitting only a part of

[3] Perhaps we should have allowed more for the distance back from the tips of the pollen tubes to the point where the callose plugs have formed in order to prevent backward "bleeding" of pollen tube protoplasm. The callose plugs in normal plants begin to form about 6 mm. back from the ends of the tubes, and occur at average intervals of 1.6 mm. A pollen tube 30 mm. long would have a newly formed callose plug about 6 mm. from its forward end and others at intervals of about 1.6 mm., with the oldest callose plug about 3.5 mm. below the top of the stigma.

the first group and in excluding all of the second group, for table II, set C, shows that all the plants obtained from these seeds were normal. This shows that the first group are n and not $(n+1)$ pollen tubes.

Table II also shows the success obtained in set B in lessening the competition among pollen tubes for fertilization of eggs. The restricted pollinations prevented the first group of pollen tubes from monopolizing the ovules, so that there were some remaining to be fertilized by the second group. The proportion of Ck plants obtained from most of these seed capsules was as high as or higher than that usually obtained in self-pollinated Ck capsules, ranging as high as 34.1 per cent in no. 24a, and giving an average of 27.6 per cent. They averaged about three times the proportions obtained in backcrosses made by the usual routine methods indicated in table I. The difference between these (table II B) and the style-excision experiments (table II C) shows that the slower group of pollen tubes are the ones which carry the extra chromosome necessary to produce the Ck $(2n+1)$ type.

Differences in proportions of Ck plants in upper and lower parts of seed capsules

There is still another set of data obtained which is in agreement in identifying the slower group of pollen tubes as made up of $(n+1)$ tubes, namely, the comparison of the upper and lower portions of the seed capsules. The pollen tubes which are first to arrive in the interior of the ovary fertilize the ovules near the top, while those which arrive later accomplish fertilization in ovules farther down. We have occasionally observed that if only a limited quantity of pollen is applied to the stigma, the lowest ovules are the ones which fail to enlarge, presumably through lack of fertilization.

Several of the capsules in these tests were not divided. No. 14a was too small to warrant a division into upper and lower half, and 13a was harvested in a state too mature to be divided. The other three capsules of set B were carefully divided into upper half and lower half and grown as separate pedigrees (table II). In every case where the capsule was divided there was an overwhelming increase in the proportion of Ck types in the lower portion of the seed capsules compared with the upper portion. Capsule 24 had 65.4 per

TABLE II

RESULTS OF RESTRICTED POLLINATIONS IN COCKLEBUR MALE BACK-CROSSES USING EXCISION OF STYLES AND DIVISION OF SEED CAPSULES INTO UPPER AND LOWER PORTIONS

PARENTS	SET B. SPARINGLY POLLINATED, STYLES NOT EXCISED									SET C. SPARINGLY POLLINATED, STYLES EXCISED					
	Capsule no.	Planting no.	Seeds planted	Plants recorded			Percentage Cocklebur			Capsule no.	Planting no.	Seeds planted	Plants recorded		
				Total	Normal	Ck	U½	L½	Whole capsule				Total	Normal	Ck
2601(1)× 260108(118)	24a U½	271011	200	188	188	0	0	.....	34.1	21a	27972	34	21	21	0
	24a L½	271012	235	205	71	134	....	65.4							
2601(4)× 260108(116)	13a	271009	146	132	120	12	.....	.....	9.1	12a	271008	19	19	19	0
	14a	271010	86	85	58	27	.....	.....	31.8						
2601(4)× 260108(118)	25a U½	27975	162	147	141	6	4.1	.....	29.2	.....	.....	.....	.....	.....	.....
	25a L½	27976	178	158	75	83	.....	52.5							
2601(5)× 260108(118)	26a U½	271013	175	167	166	1	0.6		24.9	22a U½	27973	45	41	41	0
	26a L½	271014	173	167	85	82		49.1		22a L½	27974	42	35	35	0
Totals...	.....	.....	1355	1249	904	345	1.4	56.4	27.6	.....	.....	133	116	116	0

cent Ck in the lower half and none in the upper, while capsules 25 and 26 had about half Ck types in the lower half. Thus by this method have been obtained pedigrees with twice the proportion of Ck types found in ordinary selfed capsules, or in pedigrees obtained by any other known method. This is remarkable in view of the rule that most $(2n+1)$ types are usually not transmitted through the pollen, and emphasizes the importance of a knowledge of the mechanics involved in these processes of gametophytic selection, by which we may control the proportion of $(2n+1)$ types obtained in a sample of progeny.

Order of fertilization in ovary

One may gain an idea of the extent to which the upper portion of a seed capsule is fertilized by the earliest arriving pollen tubes from an experiment conducted in 1922. The flowers of white plants were pollinated with pollen (20–60 grains) from purple plants, and repollinated after 24 hours by selfing. Thus the pollen tubes from the crossed pollen had a day's start over those of the selfed pollen, and must have reached the ovary first if they grew at all. The seedlings of the cross could easily be identified by their color, since purple is a dominant with respect to the recessive white gene. The seeds obtained from eight capsules were divided into three sections, representing upper, middle, and lower portions, and grown as separate pedigrees (table III). In the total, 92.2 per cent of the purple plants were found in the upper portion of the seed capsule, which represents the upper fourth of the total seed population, while only 6.7 per cent were found in the middle section, and only a single purple plant was obtained from seeds of the lower portion.

In another similar series of tests not tabulated here, a total of 987 plants from five seed capsules gave similar results. These five seed capsules were each divided into two parts, the upper halves containing an average of 94.5 per cent of the purple plants and the lower halves including only 5.5 per cent. Both experiments demonstrated conclusively that the early arriving pollen tubes enter the ovules situated near the top of the ovary.

Wedge as a secondary of Cocklebur

Wedge (Wd) is the only secondary of Cocklebur which has been recognized. In its breeding behavior, line 1A Wedge plants when

selfed have thrown 628 *2n* plants, 149 Wd, 8 Ck, its primary, and from one to two each of five other unrelated types. This breeding behavior indicates that Ck is its primary (2).

TABLE III

EARLY AND LATE ARRIVAL OF POLLEN TUBES IN OVARY DUE TO COUNTED POLLINATIONS, AND REPOLLINATIONS AFTER DEFINITE INTERVALS

No.	Seeds planted	Plants recorded	Region in seed capsule					
			Upper		Middle		Lower	
			P	W	P	W	P	W
22–15	102	89	17	72	.....	.....	.....	.....
	158	146	.....	.....	1	145	.....	.....
	155	148	.....	.....	.....	.....	0	148
22–16	80	78	8	70	.....	.....	.....	.....
	178	168	.....	.....	2	166	.....	.....
	227	190	.....	.....	.....	.....	0	190
22–56	122	111	38	73	.....	.....	.....	.....
	271	257	.....	.....	1	256	.....	.....
	275	253	.....	.....	.....	.....	0	253
22–62	94	89	1	88	.....	.....	.....	.....
	266	246	.....	.....	1	245	.....	.....
	284	271	.....	.....	.....	.....	0	271
22–63	101	96	6	90	.....	.....	.....	.....
	200	193	.....	.....	0	193	.....	.....
	146	136	.....	.....	.....	.....	0	136
22–64	177	160	5	155	.....	.....	.....	.....
	189	159	.....	.....	0	159	.....	.....
	179	171	.....	.....	.....	.....	1	170
22–65	93	86	1	85	.....	.....	.....	.....
	260	256	.....	.....	0	256	.....	.....
	317	311	.....	.....	.....	.....	0	311
22–55	87	74	7	67	.....	.....	.....	.....
	161	151	.....	.....	1	150	.....	.....
	167	140	.....	.....	.....	.....	0	140
Totals	.....	2979	83	700	6	1570	1	1619

No specific tests have been made of the pollen transmissibility of Ck from Wd pollen under conditions of restricted pollinations, but some data have been obtained from routine pollinations which show that Ck is present in significant proportions in the offspring of male

back-crosses of Wd. These data are included in table I, where an average of 2.7 per cent Ck was found in the offspring of the Wedge male back-crosses. These records from male back-crosses may therefore be taken as further evidence to indicate that Wedge is a secondary of Cocklebur.

Cytological evidence also indicates that Wedge is not a primary but a secondary (1), and its external and internal morphological features relate it to Ck (24).

Pollen tube growth from pollen of Wedge

Figs. 9–11 show pollen tube distribution curves of Wedge, and fig. 12 is that of a normal plant. A high proportion of the pollen of

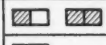
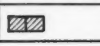
	Trisome	Disjunction of Trisome	Performance of Gametophytes	Plants Obtained if United with Normal Egg
Cocklebur Primary			Pollen tubes with slow growth	Cocklebur
			Normal pollen tubes	Normal
Wedge Secondary			Pollen tubes swell and burst near stigma	Would produce Wedge
			Normal pollen tubes	Normal
			Pollen tubes with slow growth	Cocklebur
			Aborted pollen grains	P

Fig. 13

Wedge germinates. The pollen tubes with n chromosomes may be found in the longest group constituting the forward mode. A large group of short tubes, most of them swollen or burst, is found in the region close to the stigma; in some of the tests (figs. 9, 10) a group of normal appearing tubes is found forming a small mode between these two main groups.

Fig. 13 illustrates our scheme for the chromosomes of Wd and Ck. In Ck, which is a primary, are three identical chromosomes forming a trisome, hence there are only two types of microspores possible at reduction, those with n chromosomes and those with $(n+1)$. In Wd, however, which has one member of its trisome different from the other two, four classes of microspores are possible. We think of

the odd member of the trisome of Wd as made up of the shaded doubled half, of the Ck chromosome, and, if the assortment of chromosomes were entirely at random, there would form four classes of microspores: normal-producing *n;* Wd-producing (*n*+Wd); Ck-producing (*n*+Ck); and (*n*+Wd-Ck), a class which is deficient. The *n* pollen tubes are easily identified (figs. 9–11); the (*n*+Wd) are likewise included in the large abnormal group near the stigma; the (*n*+Ck) group may be the small mode between these; and the (*n*+Wd-Ck) are represented by aborted pollen grains, not included in these tests. The assortment of chromosomes in the trisome of Wd at reduction is evidently not entirely at random, that is, 2:2:1:1, for the diagrams (figs. 10, 11) do not show half as many (*n*+Ck) pollen tubes as *n* and (*n*+Wd). Likewise the proportion of aborted pollen in Wd ranging from 6 to 10 per cent (**4**) is not as high as it should be if we assume random assortment of the chromosomes in the disjunction of this trisome. In fig. 11 the small (*n*+Ck) group is 8.5 per cent of all the normal pollen tubes which have passed or reached this point, and in fig. 10, where a similar group is found in a more retarded position, these represent 13 per cent of the pollen tubes which have passed or reached this distance. While the small groups of retarded normal-appearing pollen tubes in figs. 10 and 11 would seem to represent (*n*+Ck) tubes, it appears that this group may not always be identified, for it should be noted that in the test of fig. 9 (a companion test of fig. 10) no such second mode was observed.

If all the pollen tubes which have passed or reached the place of these small second modes (figs. 10, 11) would fertilize ovules giving rise to seeds (these groups resulting in Ck plants), there would be 73 Ck plants in a total of 786, or 9.3 per cent. This is about three and a half times in excess of the results shown in table I, where an average of 2.7 per cent Ck plants have been obtained from Wd male back-crosses in pollinations employing routine methods. However, the excess of Ck plants in table II, B over I (restricted pollinations versus routine pollinations) is in about this same proportion.

The fact that two Wedge plants were obtained in the back-crosses (table I) is rather surprising in view of the conditions shown in figs. 9–11, as well as in many other similar tests not included here. However, the proportion of Wd transmitted is only 0.3 per cent of the

total. The data in table I are not comparable with the graphs, since the latter are confined to line 1 plants and in table I the Wd plants that were back-crossed were heterozygous for other lines. The primary Pn heterozygous for certain lines when male back-crossed to these lines has produced as many as 11 per cent of Pn individuals. If only line 1 had been involved in these male back-crosses, no Pn individuals would have been expected in the offspring. The only male back-crosses of Wd involving merely line 1 in our records gave a total of 83 offspring, all normals.

It may be concluded that Wd is not transmitted through the pollen in line 1A. With the exception of the two Wd plants noted in table I when line 21 was involved, no instances of pollen transmission of Wd have been obtained.

Discussion

The successes secured in efforts to control the processes of gametophytic selection in the transmission of Ck through the pollen have been gratifying. It is possible to obtain conditions in which none of the Ck is transmitted, or to vary the conditions so that half or more than half of the seeds selected for planting will produce Ck plants. The method for obtaining no Ck plants through the pollen is to pollinate with much pollen, and, if possible, to excise the styles after the first group of pollen tubes have entered the ovary. If styles are not excised, only the seeds from the upper half of the seed capsules must be planted. The method for obtaining a maximum of Ck transmission is to pollinate sparingly (with 400–500 pollen grains) and to plant only the seeds taken from the lower half or third of moderate-sized seed capsules. Small seed capsules are often the result of severe interovular selection, in which the ovules containing $(2n+1)$ zygotes suffer a higher mortality than those containing $2n$ zygotes.

All the experiments have confirmed our interpretation regarding the identity of the groups of pollen tubes visible in the microscopic preparations. We may now predict from similar tests of other $(2n+1)$ plants, where it may be feasible to make male back-crosses using restricted pollinations with the expectation of obtaining transmission of the extra chromosome.

Since several methods have been combined in this study of the

pollen transmissibility of Ck and Wd, it may be of interest to enumerate briefly these methods as used by various investigators, where pollen tube growth conditions were involved.

Restricted versus abundant pollination

Correns (**11**) used small versus large amounts of pollen in pollinations of the dioecious plant *Melandrium*. He obtained an increase in the proportions of staminate plants when small amounts of pollen were employed and a decrease with large amounts. His explanation, based on the theory that pollen tubes carrying the staminate determining gametes grow slower than pollen tubes transmitting the pistillate determining gametes, was confirmed by many subsequent experiments (**12, 13**).

In an investigation on the number of pollen grains necessary to induce seed setting in *Mirabilis*, Naudin (**23**) was the first to make counted pollinations. Goodspeed and Davidson (**18**) counted the pollen grains and determined the minimum number necessary to obtain seeds in *Nicotiana langsdorfii* var. *grandiflora*, and Snow (**26**) used counted pollinations in a genetic study of *Matthiola*.

We have employed restricted pollinations since 1921 in a study of the pollen transmissibility of Globes (**14, 15**) by a method of counting the pollen grains. The results, which have not been published in detail, have demonstrated that the pollen tube growth phenomena involved in the transmission of Globes may be modified experimentally by the abundance of pollen on the stigma, but in *Datura* there is also a crowding effect among the ovules. As a result of this process of interovular selection, the proportions of $(2n+1)$ plants which survive are further modified.

Excision of styles

Excision of styles at various intervals after pollination was a method employed by Correns in *Melandrium* (**13**). Heribert-Nilsson (**19**) employed the method of cutting off styles in a study of "certation" in *Oenothera lamarkiana*. He compared the time required for obtaining fertilization in flowers pollinated with their own pollen with those on other flowers of the same plant pollinated with the pollen of the mutant form *gigas*, and found that the pollen tubes of the

last named form require a slightly longer period of growth to accomplish fertilization before excision than the pollen tubes from selfing. In a later investigation (**20**) he discovered by properly timed style-excisions that from pollen heterozygous for *rubrinervis* there are many more pollen tubes bearing the genes for *rubrinervis* among those which arrive at the ovary during the earliest hours of fertilization than one or two hours later.

DAVIS (**16**), using the cross *Oenothera lamarkiana* × *nanella-brevistylis*, excised the styles at various intervals after pollination and obtained in a rather limited population (total of 100 plants) ratios of 1:5 when the styles were cut off 23 hours after pollination, and a ratio of 1:2.8 when they were cut off 24 hours after pollination. Likewise SIRKS (**25**) obtained differences in the ratios of *Datura* in crosses involving the segregation of purple-armed and white-inermis in male back-crosses, by cutting off the styles at various intervals after pollination.

PROPORTIONS FOUND IN UPPER AND LOWER HALVES OF SEED CAPSULES

CORRENS (**12**) proved experimentally that the ovules in the upper part of the ovary of *Melandrium* are entered by the pollen tubes which arrive early, and introduced the methods of dividing the pedigrees into upper and lower portions. He found the proportions of staminate and pistillate plants derived from the seeds of these regions of the ovary consistent with his theory that the staminate determining pollen tubes are slower than the pistillate determining pollen tubes.

Similar methods have been employed by JONES (**21**), BRINK (**5**), and other breeders of maize, using styles left long, compared with styles cut short, and counting the proportions in upper and lower parts of the ears separately, etc. These methods are fully discussed by JONES, and while some of the factors involved seem to be similar to ours, they are not further cited and discussed here, because the conditions are not entirely comparable with the present problem. The ear of maize is an inflorescence of many one-ovuled pistils, and we are concerned with single flowers having compound pistils with a common style and stigma and many ovules.

POLLEN TUBE DISTRIBUTIONS DURING GROWTH

EAST and PARK (17) made the first attempts to analyze a pollen tube population by the use of histological methods, using serial paraffin sections, stained and reconstructed so that the distribution of the tubes could be plotted. We have adopted a similar method, following a more rapid and accurate special technique of dissection, and have plotted pollen tube distribution curves in a number of investigations. It has seemed advisable, therefore, to test the validity of this rapid technique in the study of pollen tube growth, by employing this new method together with as many other methods as possible in an attack on an individual problem.

The experience of cotton breeders in excising styles has not given support to a theory based on differential growth rate of pollen tubes. KEARNEY and HARRISON (22) failed to obtain significant differences in the proportion of hybrids and selfs when they employed pollen mixtures of Upland and Pima cotton, or when they used excision of styles; they also failed to find differences in the proportions between the upper and lower halves of seed capsules, although their experience indicated that there must be some kind of inequality in the ability to accomplish fertilization by the two kinds of pollen.

In the face of the results of KEARNEY and HARRISON, it would seem to be desirable to have every method used in such investigations thoroughly tested. In this study of pollen transmissibility we have shown that there is complete agreement in our results from the employment of four of these methods of investigation, and there can be no question about the fact that the pollen tubes carrying the extra Ck chromosome have a slower rate of growth than normal ones.

In the study of the pollen of heterozygous plants, our pollen tube counting method has a distinct advantage in enabling us to distinguish between the following types of pollen tube growth conditions which may result in the complete or partial failure of pollen transmission when gametophytic selection is involved: (1) complete failure of half of the pollen to germinate on the stigma, as in the case of polycarpic (10); (2) delayed germination of half of the pollen, resulting in bimodal distributions; (3) differential growth rate between two classes of pollen tubes also resulting in bimodal distributions, the last two conditions being distinguishable by comparing

pollen tube growth tests made after different intervals of time; (4) complete elimination of half of the pollen tube population through bursting as in Wd, and the question of whether or not this is preceded by a delay in germination; (5) partial elimination of half of the pollen tube population through bursting of pollen tubes, as we have found in the tricarpel race of *Datura* (**8**).

Summary

1. Pollen tube distributions were plotted from 12-, 16-, and 18-hour tests, using the pollen of the primary ($2n+1$) Cocklebur germinated and grown on the pistils of $2n$ plants. These tests were compared with similar tests of pollen tube growth of normal plants in line 1A.

2. Further evidence is given from the results of ordinary male back-crosses of Wedge to four lines, indicating that the latter is a secondary ($2n+1$) type related to Cocklebur. Pollen tube distributions obtained from the pollen of Wedge are also plotted.

3. The pollen tube distribution curves indicate that Cocklebur may be transmitted through the pollen under conditions in which the processes of gametophytic selection are controlled experimentally. Likewise, the distribution curves indicate that Wedge may not be transmitted through the pollen in line 1A.

4. The correctness of the interpretations gained from pollen tube distribution curves was tested genetically in the case of Cocklebur, by combining three other experimental methods: the effects of restricted pollinations; the effects of excision of styles; and the separation of the seeds in the lower half of seed capsules from those in the upper half. Results from all experimental methods confirmed the interpretations gained from a study of pollen tube distribution curves.

5. The various methods which have been used by investigators at different times in a study of the effects of gametophytic selection are briefly discussed and compared.

6. The pollen tube distribution curves obtained from suitable tests enable us to recognize processes of gametophytic selection, and have the further advantage of enabling us to distinguish between at least five different conditions, all of which may lead to the complete

or partial elimination of some of the classes of male gametophytes, resulting in modified ratios in the progeny.

7. The processes of gametophytic selection involved in the pollen transmission of Cocklebur may be controlled through a wide range. A threefold increase may be obtained by restricted pollinations, and a further increase, up to 65 per cent Cocklebur plants, may be obtained in a progeny by selecting the seeds to be planted from the lower parts of seed capsules, while complete non-transmission of Cocklebur may be obtained by properly timed excisions of styles.

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. V

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 408

EARL EDWARD SHERFF

(WITH PLATES IV, V)

COREOPSIS BORIANIANA Schz. Bip. *ex* Schweinf., Verhandl. Zool.-Bot. Ges. Wien **18**:684. 1868; *C. borianiana* var. *cannabina* Schz. Bip. *loc. cit.*; *C. guineensis* O. & H. *in* Oliver Fl. Trop. Afr. **3**:390. 1877; *C. chrysopterocarpa* Chiov., Ann. Bot. Roma **9**:75. 1911.

SCHULTZ BIPONTINUS based his *C. borianiana* upon (1) *Boriani* 42, in the Fesoglu, 1839 and (2) *Schweinfurth* 439, Matamma, Oct. 9, 1865. He based his var. *cannabina* upon a larger specimen by *Schweinfurth*, no. 585, also collected at Matamma in the middle of October, 1865. The species proper, as represented by the *Boriani* specimen (I have seen SCHULTZ BIPONTINUS' type fragment at Paris), has leaf segments 3–6 mm. wide, while the var. *cannabina* has leaf segments 0.8–2.2 cm. wide. These distinctions appear to rest, however, upon different stages of development. Thus, for example, a specimen of *Schweinfurth* 439 in SCHULTZ BIPONTINUS' own herbarium (in Herb. Par.) has, above, the narrow leaf segments described for the species, and, below, one leaf with the broader segments described for the variety and matching the leaves of *Schweinfurth* 585 (Herb. Berl.). In fact, SCHULTZ BIPONTINUS himself had labeled this specimen var. *cannabina*.

With these considerations in mind, I have reduced the var. *cannabina* to synonymy with *C. borianiana* proper.

OLIVER and HIERN (*in* Oliver *loc. cit.*) based their *Coreopsis guineensis* primarily upon a plant by *C. Barter*, collected at Nupe, Nigeria (Herb. Kew). The *Barter* plant is purely *C. borianiana*, as are also many other plants secured by various collectors more recently and referred in herbaria to *C. guineensis*.

Some time ago I was permitted, through the kindness of Dr. GIOVANNI NEGRI of Florence, Italy, to examine the type of *C. chrysopterocarpa* Chiov. (*Pappi* 7733, alt. 1000 m., Baza-Cunama-Barentù,

Eritrea, Nov. 28, 1908, in Herb. R. Inst. Bot. Florence). It proved to be identical with *C. guineensis* O. & H. and hence with *C. borianiana*.

Specimens examined: *C. Barter* 933, Nigeria, 1857–1859 (Herb. Berl.; Herb. Gray); *Boriani* 42, in the Fesoglu, northeastern Africa, 1839;[1] *Aug. Chevalier* 2083, Koulitsoro, Sudan, 1899 (Herb. Berl.; Herb. Bruss.); *idem* 9633, Massenya (Massenia), Bagirmi (Baguirmi), Sudan, 1902–1904 (Herb. Berl.); *Dr. Kersting* 14, alt. 350 m., Trógode, Kerkri, Togo, Oct. 28, 1897 (Herb. Berl.); *idem* 422, alt. 400 m., Sokodé-Basari, Togo, October, 1901 (Herb. Berl.); *Lécard* 290, Sénégal (Herb. Bruss.); *C. Ledermann* 5243, growing 1–1.5 m. high, alt. 380 m., between Tschamba and Doreba, Kamerun, Sept. 18, 1909 (Herb. Berl., 2 sheets); *A. Pappi* 7733, alt. 1000 m., Baza-Cunama-Barentù, Eritrea, Nov. 28, 1908 (Herb. Flor.; type of *Coreopsis chrysopterocarpa* Chiov.); *Dr. T. Pfund*, Dar-Fur, 1876 (Herb. Berl.); *idem* 336, Takari on the Rahad, Kordofan, July, 1875 (Herb. Berl., 2 sheets); *Dr. Rowland*, common in savannahs, western Lagos, 1893 (Herb. Berl., 2 sheets); *F. Schroeder* 74, alt. 350 m., Sokodé Farm, Togo, Oct. 19, 1900 (Herb. Berl., 2 sheets); *Dr. G. Schweinfurth* (Flora of Gallabat no. 439), Matamma, Gallabat (Galabat) region, northwestern Abyssinia, middle of October, 1865 (Herb. Schz. Bip. *in* Herb. Par.); *idem* (Flora of Gallabat no. 585), *eodem loco et tempore* (Herb. Berl.; type material of var. *cannabina* Schz. Bip.).

COREOPSIS BORIANIANA **multiplex** var. nov.—A specie involucri bracteis exterioribus biserialibus 18–22, plerumque 1.4–1.8 cm. longis quam interioribus saepe longioribus differt.

Specimens examined: "*Graf. Zech.* 168," Kete Kratschy, Togo, Oct. 21, 1898 (Herb. Berl.; type).

COREOPSIS PACHYLOMA O. & H. *in* Oliver Fl. Trop. Afr. **3**:391. 1877; *Coreopsis involucrata* Schz. Bip. *in* Walpers Repert. **6**:163. 1846 (*non* Nutt.); *Verbesina involucrata* (Schz. Bip.) A. Rich. Tent. Fl. Abyssin. **1**:409. 1847; *Coreopsis callosa* Schz. Bip. *in* Schweinf. *et* Aschers. Enum. 284.

SCHULTZ BIPONTINUS definitely published his *Coreopsis involu-*

[1] The type was cited for the Herbarium of the Museum of Vienna, and doubtless I examined it there some years ago. I have had at hand more recently, however, the type fragment from SCHULTZ BIPONTINUS' herbarium (*in* Herb. Par.).

crata in 1846 with a valid description. Later, to avoid conflict with NUTTALL'S *C. involucrata*, he renamed his own species *C. callosa*.

My recent study of the types and other specimens of *Coreopsis pachyloma* O. & H. and of *C. involucrata* Schz. Bip. showed them to be identical. OLIVER and HIERN clearly were unfamiliar with the status of *C. involucrata* Schz. Bip. and its equivalent, *C. callosa* Schz. Bip., for they published these two names (Oliver Fl. Trop. Afr. 3:392. 1877) as of "unknown species of *Coreopsis*" and treated them both as *nomina nuda*.

In recent decades *Coreopsis involucrata* Nutt., a species of North America, has become generally accepted as belonging in *Bidens*. In that genus it is *B. involucrata* (Nutt.) Britt. under the Vienna Rules, or *B. polylepis* Blake under the so-called American Rules. Thus *Coreopsis involucrata* Schz. Bip. is left as the next name for the species in *Coreopsis* that was later named *C. pachyloma* and, if the rules as to synonymy in the Vienna Code (versus the American) were to be followed, would take precedence over *C. pachyloma* O. & H.

Specimens examined: *Rüppell*, between Temben and Siemen, Abyssinia, June or July, 1832 (Herb. Par.; type of *C. involucrata* Schz. Bip.); *Wilhelm Schimper* (*iter abyssin. sect.* V) 15, Abyssinia, 1853 (Herb. Berl.; Herb. Deless.; Herb. Par.); *idem* (*iter abyssin. sect* V) 933 *pro parte*, Abyssinia (Herb. Par.); *Dr. Steudner*, Gaffat near Debra Tabor, Abyssinia, May 10, 1862 (Herb. Berl.).

COREOPSIS PACHYLOMA **inanis** var. nov.—A specie achaeniis etiam primo exaristatis differt.[2]

Specimens examined: *Wilhelm Schimper*, no. 933 *pro parte*, Abyssinia, *commun.* 1853 (Par.).

COREOPSIS OLIGOFLORA **robusta** var. nov.[3]—A specie involucri bracteis exterioribus late oblongis vel ovatis saepius 2.5–4 mm. latis apicaliter obtusissimis differt.

Specimens examined: *Büttner* 408 (Herb. Berl.; type); *P. Pogge* 1292, Mukenge tract (*campine*), western Africa, Apr. 13, 1882 (Herb. Berl.).

[2] The American Code is followed in this instance. As already implied, the Vienna Code would require *C. involucrata* var. *inanis* for the name here.

[3] The name *robusta* was used for this variety on a herbarium label by Dr. OTTO HOFFMANN, in connection with KLATT'S later (and philologically better) name *C. oligantha*. I do not seem, however, to have noticed the name in print.

COREOPSIS PRESTINARIAEFORMIS **incisa** var. nov.; *C. heterocarpa* Chiov., Ann. Bot. Roma **9**:75. 1911 (*pro parva parte*).—A specie foliis acriter et perspicue bipinnatisectis segmentis principalibus oblongo-lanceolatis differt.

Specimens examined: *Dr. Emilio Chiovenda* 1090, very common, Gondar, Province of Amhara, Abyssinia, Jul. 26, 1909 (Herb. Flor.); *Dr. Ellenbeck* (*Exped. Baron von Erlanger*) 1436, growing 1 m. high, alt. 2600 m., Djafa, Arussi Galla, Gallaland, Jul. 21, 1900 (Herb. Berl.; type).

In July–September, 1909, CHIOVENDA collected in the Province of Amhara, Abyssinia, seven plants which he assumed to be the same. Of these I have examined six, and find five (nos. 970, 1148, 1869, 1953, and 1954) to be *C. prestinariaeformis* Vatke.[4] A sixth one (no. 1090) has noticeably bipinnatisect foliage, such as is found commonly in *C. prestinaria* Schz. Bip., and easily merits varietal distinction.

Unfortunately, CHIOVENDA seems to have been unfamiliar with *C. prestinariaeformis* Vatke (the types of which are at Berlin). He founded his *C. heterocarpa* upon these seven specimens. From alphabetical and numerical considerations his no. 1090 (my var. *incisa*) was cited first but his description clearly was based principally upon the other specimens, that is, those of *C. prestinariaeformis* proper. Hence it is impossible of course to utilize here the name *heterocarpa*. I have chosen, therefore, the entirely new name *incisa*.

Bidens chaetodonta nom. nov.; *Coreopsis abyssinica* Schz. Bip. *in* Walpers Repert. **6**:163. 1846; *Verbesina abyssinica* (Schz. Bip.) A. Rich. Tent. Fl. Abyssin. **1**:409. 1847; *Prestinaria* (*Steppia*) *abyssinica* Schz. Bip. *in* Herb. Schimp. Abyssin. *sect.* I, n. 332 *ex* O. & H. *in* Oliver Fl. Trop. Afr. **3**:389. 1877.

Specimens examined: *Dr. Emilio Chiovenda* 3151, Province of Amhara, Abyssinia, Dec. 7, 1909 (Herb. Flor., 2 sheets); *Quartin Dillon*, Abyssinia (Herb. Par.); *A. Petit*, Abyssinia (Herb. Berl.; Herb. Par.); *idem*, Chiré, Abyssinia (Herb. Berl.); *Wilhelm Schimper* 332, in upper region, northern side of Mt. Scholoda, Abyssinia, Oct. 3, 1837 (Herb. Berl.; Herb. Par., 5 sheets; type material).

[4] The type specimens of which (Herb. Berl.) have rather immature capitula and fairly slender achenial aristae (*cf.* O. & H. *in* Oliver Fl. Trop. Afr. **3**:389. 1877: "filiform acute aristae"). *Chiovenda's* specimens are taller, more robust, and have many mature achenes with the characteristically lanceolate-subulate aristae such as are found also in *C. macrantha* Schz. Bip., etc.

BIDENS CHAETODONTA var. **glabrior** (O. & H. *in* Oliv.) comb. nov.; *Coreopsis abyssinica* var. *glabrior* O. & H. *in* Oliver Fl. Trop. Afr. 3:389. 1877.

Specimens examined: *Dr. Ellenbeck* 1566, alt. above 2000 m., Akaki, South Schoa, Abyssinia, Aug. 13, 1900 (Herb. Berl.); *idem* 1592, alt. about 2300 m., Adis Abeba, Schoa, Oct. 3, 1900 (Herb. Berl.); *idem* 1614, alt. 2300 m., Akaki, Dec. 26, 1900 (Herb. Berl.); *A. Pappi* 5205, alt. about 2610 m., Mt. Zagher, Eritrea, May 20, 1902 (Herb. Flor.); *A. Petit*, Chiré, Abyssinia (Kew; type); *idem* 138, *eodem loco*, August, 1840 (Herb. Par.); *idem* 587, *eodem loco* (Herb. Par.).

Bidens dielsii nom. nov.;[5] *Coreopsis chrysantha* var. *simplicifolia* Vatke, Linnaea 5:500. 1875; *C. simplicifolia* (Vatke) Engler Hochgeb. Fl. Trop. Afr. 435. 1892.

Specimens examined: *Oscar Neumann* 135, alt. 2800 m., mountain meadows, Gardulla, Gallaland, Jan. 14, 1901 (Herb. Berl.); *Dr. Ragazzi* 1311, Dallo Scioa, Tropical East Africa, 1887 (Herb. Flor., 2 sheets); *Wilhelm Schimper*, alt. 7000 ft., on mountains at Dewari, Abyssinia, Oct. 2, 1863 (Herb. Berl.; 2 type sheets).

It is difficult when studying the type collections to understand why VATKE referred this plant to a variety of his *Coreopsis chrysantha*, even though they were both collected by *Schimper* and on the same day. OLIVER and HIERN (*loc. cit.*) stated: "This variety of VATKE appears to constitute a distinct species. . . . ." Later, ENGLER very properly elevated it to specific rank. The species is better placed, however, in *Bidens*.

Bidens vatkei nom. nov.; *Coreopsis chrysantha* Vatke, Linnaea 39:499. 1875 (*non* L. Sp. Pl. edit. II:1282. 1763).

Specimens examined: *Dr. Emilio Chiovenda* 1699, Gondar, Dembià, Province of Amhara, Abyssinia, Aug. 28, 1909 (Herb. Flor.; *forma foliis majoribus, foliolis latioribus terminali usque ad 3.5 cm. lato*); *idem* 1992, near Asosò, Dembià, Sept. 13, 1909 (Herb. Flor.); *Wilhelm Schimper*, alt. 7000 ft., Valley of Repp River, Dewari, Abyssinia, Oct. 2, 1863 (Herb. Berl., 2 sheets of type material).

[5] The type material of this and many other species was lent to me for study several years ago through the kind permission of Dr. LUDWIG DIELS, Director of the Berlin Botanical Garden. Because of the existence already of *Bidens simplicifolia* Wright, however, the adoption here of a new trivial name becomes compulsory. In this connection it is a pleasure to commemorate Dr. DIELS' name and thus help to repay in small part the great debt that the science of botany owes him for this invaluable aid.

Bidens rueppellii (Schz. Bip.) comb. nov.; *Coreopsis rueppellii* Schz. Bip., Walpers Repert. **6**:163. 1846; *Verbesina ruppellii* A. Rich. Tent. Fl. Abyssin. **1**:410. 1847; *V. rueppellii* (Schz. Bip.) A. Rich. *ex* Vatke Linnaea **39**:499. 1875.

Specimens examined: *Dr. Emilio Chiovenda* 2930, Debarek, Semien, Province of Amhara, Abyssinia, Nov. 29, 1909 (Herb. Flor., 2 sheets); *idem* 2906, Darà, Uogherà, Province of Amhara, Nov. 28, 1909 (Herb. Flor.); *Rueppell*, Siemen, Abyssinia, August or September, 1832 (type, Herb. Par.; a mere fragment but decisively distinctive); *Wilhelm Schimper*, Abyssinia (Herb. Par.); *idem* 706*B*, Abyssinia, 1854 (Herb. Deless.; Herb. Par.).

A hitherto but little known species. VATKE (*loc. cit.*) doubtfully referred it to his *Coreopsis chrysantha* (my *Bidens vatkei*) while OLIVER and HIERN (Oliver Fl. Trop. Afr. **3**:388. 1877) stated that it was unknown to them. The achenes are black, exalate, and match those of many other species of *Bidens*.

BIDENS BITERNATA var. **glabrata** (Vatke) comb. nov.; *B. abyssinica* var. *glabrata* Vatke, Linnaea **39**:500. 1875.

VATKE's variety *glabrata* was described as having a glabrous stem and was based upon a plant by *Wilhelm Schimper*, no. 105, Gaha Meda near Dschadscha, Abyssinia, 1854. VATKE's variety appears to have been completely overlooked by subsequent workers. It is the glabrous-stemmed variety of the plant which was named *Bidens abyssinica* by SCHULTZ BIPONTINUS and later renamed *B. chinensis* var. *abyssinica* O. E. Schz., also *B. biternata* var. *abyssinica* Sherff. Under the Vienna Code the name *glabrata* must be taken up for the varietal rank.[6]

The hairy-stemmed form of this variety has not been generally distinguished by me in my herbarium determinations thus far, but is the form described originally as *Bidens abyssinica* Schz. Bip. (Walpers Repert. **6**:167. 1846) and later as *B. quadriseta* Hochst. (*in* Herb. Schimp. Abyssin. *ex* O. & H. *in* Oliver Fl. Trop. Afr. **3**:393. 1877). As the hairy-stemmed plants appear to be fairly constant as well as of strikingly different appearance, they may be set off under the var. *glabra* as f. **abyssinica** (Schz. Bip.) comb. nov.

[6] Certain earlier varietal names (*B. abyssinica* var. *quadriaristata* Hochst. *ex* Schweinf. Beitr. Fl. Aethiop. 142. 1867; var. *incisifolia* Hochst. *in herb.*, etc.) were not validly published.

Bidens microphylla nom. nov.; *Coreopsis pulchella* O. Hoffm., Engler Bot. Jahrb. **38**:204. 1906; Engler Pflanzenw. Afr. **1**: 155. *tab.* 124. 1910.

Specimens examined: *Dr. Ellenbeck* 1370, alt. 3000 m., on rocks, Abulkasin, Arussi Galla, southern Abyssinia, Jul. 16, 1900 (Herb. Berl.; type).

Bidens setigera (Schz. Bip.) comb. nov.; *Coreopsis setigera* Schz. Bip., Walpers Repert. **6**:163. 1846; *Chrysanthellum* (*Microlecane*) *abyssinicum* Schz. Bip., *loc. cit.* 171; *Verbesina lineata* A. Rich. Tent. Fl. Abyssin. **1**:410. 1847; *Coreopsis abyssinica* var. *bipinnato-partita* Chiov. *ex* Pirotta Fl. Colon. Eritrea **1**:185. 1904.

Specimens examined: *Dr. Emilio Chiovenda* 2051, Gondar, Province of Amhara, Abyssinia, Sept. 13, 1909 (Herb. Flor.); *idem* 2699, Province of Amhara, Oct. 27, 1909 (Herb. Flor.); *idem* 2750, Gondar (Herb. Flor.); *idem* 3253, Division of Tigré, Abyssinia, Dec. 11, 1909 (Herb. Flor.); *A. Pappi* 559, alt. about 2000 m., Adi Quala, Saraè, Eritrea, Oct. 23, 1902 (Herb. Flor.); *idem* 3871, Eritrea, Mar. 2–10, 1902 (Herb. Flor.); *idem* 4101, alt. about 600 m., Valle Damas, Ocule Cusai, Eritrea, Apr. 14, 1893 (Herb. Flor.); *idem* 4209, Dongollo near Ghinda, Eritrea, Mar. 12, 1902 (Herb. Flor.); *Dr. A. Ragazzi* 3360/3860, Scioa, Tropical East Africa, Oct. 10, 1886 (Herb. Flor.); *Rüppell*, Abyssinia, 1831–1832 (Par.; type); *Wilhelm Schimper* (*iter abyssin. sect* II) 766, along edges of groves in valleys near Adoa, Abyssinia, Nov. 12, 1838 (Herb. Par.; type of *Chrysanthellum abyssinicum* Schz. Bip.); *Sing Scotti*, Ghinda, Eritrea, 1893 (Herb. Flor.).

Bidens magnifolia nom. nov.; *Coreopsis frondosa* O. Hoffm., Engler Pflanzenw. Ost-Afr. *C*.414. 1895. Pl. IV.

Specimens examined: *Albers* 204, growing 2–3 m. high at alt. 1600 m., Kwai, German East Africa, Oct. 22, 1899 (Herb. Berl.); *Karl Braun* 671, growing over 1 m. high, Amani, German East Africa, May 12, 1905 (Herb. Berl., 2 sheets); *idem* 2682, Lutindi, East Usambara, German East Africa, Aug. 15, 1909 (Herb. Berl.); *Dr. J. Buchwald* 263, growing 2.5 m. high, Usambara, Dec. 20, 1925 (Herb. Berl.); *C. Holst* 2252, growing 2–2.5 m. high, Kwa Kiniarri, Nderema, Tropical East Africa, Feb. 23, 1893 (Herb. Berl.; type); *idem* 9149*a*, alt. 1600 m., high forest clearings, Kwa Mstuza, Tropical East Africa, Aug. 17, 1893 (Herb. Berl.); *Dr. Houy* (*Exped. Hans Meyer*) 1190, growing 1–2 m. high, Nsogiro Mountains, Ussagara, District of Kilossa, Morogoro, German East Africa, November–December, 1911 (Herb. Berl.); *Münzner* (*Exped. Capt. Fromm*) 35, growing 2 m. high along brook, Langenburg, German East Africa, Jul. 30, 1908 (Herb. Berl.); *Preuss* 689, Buea, Kamerun, 1891 (Herb. Berl.); *Dr. Franz Ludwig Stuhlmann* 8912, alt. 1400 m., German East Africa, Oct. 20, 1894 (Herb. Berl.); *idem* 9265, German East Africa, Nov. 20, 1894 (Berl.);

Warnecke 63, growing 1.5 m. high, alt. 700–900 m., Amani, German East Africa (Herb. Berl.); *Albrecht Zimmermann* 63, alt. 900 m., Amani, December, 1902 (Herb. Berl., 2 sheets).

Bidens ternata (Chiov.) comb. nov.; *Coreopsis ternata* Chiov., Ann. Bot. Roma **9**:74. 1911.

Specimens examined: *Dr. Emilio Chiovenda* 2581, very rare, Valley of Scintà above Asosò, Dembià, Abyssinia, Oct. 17, 1909 (Herb. Flor.; type).

Bidens rotata sp. nov.; *Coreopsis buchingeri* Schz. Bip. *ex* Schweinf. *et* Aschers. Enum. 284. (*nomen*).-Herba perennis, erecta, 6–10 dm. alta, supra ramosa, caule infra fere glabra supra hispida. Folia opposita subtenuiter petiolata petiolis planis hispidociliatis basaliter in poculum circ. 1 mm. altum connatis usque ad 2 cm. longis, petiolo adjecto saltem 4–7.5 cm. longa, tripartita foliolis membranaceis apicaliter subacuminatis ad medium usque ad 2.7 cm. latis nunc ovatis nunc lanceolatis supra viridibus ac subglabris infra pallidis ac papillato-hispidis margine ciliatis ac acerrime serratis dentibus mucronato-setigeris. Capitula tenuiter pedunculata pedunculis hispidis usque ad 18 cm. longis, radiata, pansa ad anthesin circ. 3.5 cm. lata et 1–1.2 cm. alta. Involucri bracteae exteriores 10–13, lineari-subspathulatae, pallidae, dorso subglabrae vel aegre adpresso-hispidae, margine spinuloso-ciliatae, apice nitido callosomucronatae, 1–1.6 cm. longae et 1–2 mm. latae saepe interiores ovato-lanceolatas dorsaliter hispidas apicaliter ciliatas paulo superantes. Flores ligulati 12, aurei, ligula anguste oblongo-elliptici, apice 2-3-denticulati, circ. 1.5 cm. longi et circ. 4–5 mm. lati; tubulosi superne aurantiaci, sicci ad medium plus minusve tumidi vel fracti vel articulati. Achaenia plana, lineari-oblonga, nigra, unaquaque facierum circ. 4-sulcata et superne adpresso-setosa setis erectis, marginibus erecto-setosa, margines versus tenuia sed non vere alata, corpore 4–6 mm. alta et 1–1.2 mm. lata, apice erecte setosa ac biaristata aristis tenuibus stramineis sursum hispidis circ. 2 mm. longis.

Wilhelm Schimper (*itin. abyssin. sect. V, n.* 706*a*, *edit. Buchinger ann.* 1854) in Abyssinia (type in Herb. Paris).

The type is in SCHULTZ BIPONTINUS' private herbarium at Paris. By him it had been given the name *Coreopsis buchingeri*, which name has appeared occasionally in literature but without description. The

closest ally is *Bidens rueppellii*. *B. rotata* appears to differ, however, in having more glabrous stems, different foliage, larger involucral bracts, and larger achenes. The slender rays, regularly 12, offer a fanciful resemblance to the spokes of a wheel, whence the trivial name.

BIDENS STEPPIA **leptocarpa** var. nov.—Herba 1–1.5 m. alta. Capitula pansa ad anthesin 5–6.5 cm. lata; bracteis exterioribus 8–10, circ. 9–14 mm. longis et 1.2–2 mm. latis, quam interioribus oblongo-ovatis paulo longioribus; floribus ligulatis plerumque 8, circ. 2.5–3 cm. longis. Achaenia anguste oblongo-linearia, plana, nigra, unaquaque facierum circ. 8-sulculata, marginibus apiceque erecto-ciliata, faciebus praecipue supra plus minusve erecto-setosa, exalata, corpore 7–10.5 mm. longa et 1.1–1.3 mm. lata, biaristata aristis erectis stramineis tenuibus sursum hispidis circ. 2 mm. longis.

Specimens examined: *Braun* 5505, growing 1 m. high, Niamutukusja Nsira, District of Bukoba, German East Africa, June 15, 1913 (Herb. Berl.); *Ad. Stolz* 729, growing 1.5 m. high at alt. 1350 m., Langenburg, German East Africa, May 26, 1911 (Herb. Berl., type; Herb. Deless., 2 cotype sheets); *Dr. Franz Ludwig Stuhlmann* (*Exped. Emin Pascha*) 4156, Nuansa, Tropical East Africa, May 20, 1892 (Herb. Berl.).

BIDENS STEPPIA var. **ambacensis** (Hiern) comb. nov.; *Coreopsis ambacensis* Hiern Cat. Welw. Afr. Pl. **3**:586. 1898; *Bidens ambacensis* (Hiern) Sherff, BOT. GAZ. **59**:309. 1915.—The type was collected by *Welwitsch*, no. 3272 *pro parte*, in marshy places on the left bank of the Caringa River, Ambaca, western Africa, June, 1855.[7] Six of the eight heads present were in fruit. The achenes are 5–7 mm. long and 0.8–0.9 mm. wide. Aristae are lacking, but in their place are two short teeth. The general habit of the plants is that of the broader-achened *Bidens steppia*, a species which *Welwitsch* likewise collected (no. 3531, Herb. Brit. Mus.; Herb. Kew: no. 3532, Herb. Brit.; Herb. Kew; Herb. Par.).

On transferring *Coreopsis ambacensis* to *Bidens* in 1915, I was unable to refer it to *Bidens steppia*, because of *B. steppia's* normally larger achenes and the fact that transitional forms had not been studied. The finding of the foregoing var. *leptocarpa*, however, also

[7] Type in Herb. Brit. Mus. Nat. Hist. A specimen at Kew bears the same number but is *Bidens grandis* Sherff, a very different species.

of several somewhat intermediate forms (as to achenial measurements) shows that *Coreopsis ambacensis*' claim to distinction is at best of only a varietal nature and rests upon the narrower, exaristate achenes.

BIDENS HOLSTII var. **rupestris** comb. nov.; *B. rupestris* Sherff, BOT. GAZ. **76**:144. 1923.—A comparison of the type of *B. rupestris* with the type and other authentic specimens of *B. holstii* shows that the only essential difference is in the achenes. In *B. holstii* these are normally exaristate or at best weakly aristate with upwardly directed bristles. In *B. rupestris* they are definitely aristate with usually 1–3 retrorse barbs.

Bidens cirsioides sp. nov. (Pl. V).—Herba elata, verisimiliter perennis, glaberrima, pallida, 8–15 dm. alta, caule subtereti glaucescenti valde ramoso ramis elongatis plus minusve simplicibus. Folia petiolata petiolis planis anguste alatis basaliter dilatatis 0.5–2 cm. longis, petiolo adjecto usque ad 1.5 dm. longa, pinnatim 3 (−5)-partita, foliolis oblonge linearibus vel lineari-lanceolatis crassiusculis nunc perspicue acriterque dentatis dentibus elongatis saepe inflexis et saepe setigeris nunc setis pro dentibus munitis infra albescentibus margine saepe revolutis terminali usque ad 12 cm. longo et 2.5 cm. lato lateralibus minoribus. Capitula numerosa corymboso-paniculata, tenuiter pedicellata pedicellis hispidis usque ad 1 dm. longis, radiata, pansa ad anthesin 4–5 cm. lata et 8–11 mm. alta. Involucri bracteae exteriores 16–24, subbiseriales, lineares, glaberrimae, apicem versus angustatae apice acriter calloso-mucronatae 8–10 mm. longae et 0.5–1 mm. latae; interiores late lanceolatae dorso ventreque apicaliter pubescentes alibi glabrae saepe paulo breviores. Flores ligulati plerumque 12, flavi, ligula lineari elliptici, paucistriati, apice subacuto vix denticulati, 1.8–2.3 cm. longi et circ. 3–4 mm. lati. Achaenia plana, lineari-oblonga, nigra, exalata, unaquaque facierum circ. 6-sulcata, faciebus marginibus valde erecto-setosa, corpore circ. 5 mm. longa et circ. 1–1.1 mm. lata, apice erecto-setosa ac biaristata aristis erectis tenuibus stramineis sursum hispidis 2–3 mm. longis.—Habitu specimen siccum foliis acriter dentatis nonnullis speciebus *Cirsii* simile.

Specimens examined: *C. Ledermann* 5440, growing 0.8–1.5 m. high at alt. of 1200–1400 m., Gendero, Pass Tchape, Kamerun, Oct. 5, 1909 (type in Herb. Berl.).

BIDENS PILOSA var. MINOR **umbrosa** f. nov.—Herba gracilis. Folia valde membranacea, 3–5-partita foliolis ovatis vel rhomboideo-ovatis, paucidentatis. Capitula minute radiata, pansa ad anthesin tantum circ. 6–7 mm. lata et 5–6 mm. alta. Flores ligulati circ. 4 vel 5, flavidi, circ. 4 mm. longi. Achaenia circ. 18–24, linearia, nigra, biaristata, 6–10 exteriora clavata atro-rubida corpore tantum circ. 4–5 mm. longa aristis demum saepe caducis, interiora nigra magis attenuata corpore 9–14 mm. longa.

Specimens examined: *Christian J. W. Schiede*, in shady places between Las Trojes and Hacienda de la Trinidad, State of Michoacan, Mexico, October (Herb. Berl., type and 2 cotype specimens).

BIDENS BIDENTOIDES var. **mariana** (Blake) comb. nov.; *B. mariana* Blake, Rhodora **31**:88 and fig. 1. 1929.—In 1926, Dr. S. F. BLAKE submitted his specimens no. 9698 and 9703 to me for study. He had suspected them of representing a new species. Notwithstanding this, I felt compelled at that time to refer them to *Bidens bidentoides* (Nutt.) Britt. Later BLAKE published his *B. mariana*, based upon these and certain other plants. This has naturally led to a careful reinvestigation of the entire matter on my part. Especially has a survey of the closely related *B. hyperborea* and *B. connata*, together with their several varieties, been found helpful. In this way it became evident at once that *B. mariana* differs from *B. bidentoides* to about the same extent as, for example, *B. connata* var. *fallax* differs from *B. connata* var. *typica*. To recognize *B. mariana* as a species, therefore, would immediately (if consistency is to be sought) entail an elevation of numerous forms that FASSETT, FERNALD, FERNALD and ST. JOHN, and other writers (myself among them) have regarded as varieties. It has seemed wiser to adopt the alternative course and reduce *B. mariana* to varietal rank.

Bidens neumannii sp. nov.—Herba perennis, subsimplex, saltem 3 dm. alta, suberecta e radice lignescenti, breviter tomentoso-hispida, caule subtereti, internodiis medianis quam foliis brevioribus, inferioribus plurime tantum 5–12 mm. longis. Folia opposita, parce petiolata petiolis planis circ. 4 vel 5 mm. longis, omnino circ. 2.7–4 cm. longa et 1.1–1.7 cm. lata, indivisa, anguste oblongo-ovata, marginibus regulariter acriterque serrata dentibus indurato-apiculatis, apicaliter subacuminata. Capitula non numerosa (6 in unico speci-

mine observata), corymboso-paniculata pedicellis 1–7 cm. longis, radiata, pansa ad anthesin ±2.8 cm. lata et circ. 7–8 mm. alta. Involucri externe hispidi bracteae exteriores 8–16, lineares, inferne saepe sensim angustatae, apice acriter indurato-mucronatae, demum circ. 6–8 mm. longae; interiores lanceolatae ventraliter nitidae et non nisi ad summam hispidae, paulo vel fere dimidio longiores. Flores ligulati circ. 10, saturate flavi, ligula elliptico-oblongi, apice bidenticulati, circ. 1.2–1.4 cm. longi. Paleae nitido-stramineae, lineari-oblongae, apice subabrupte mucronatae, nervo mediano perspicuae, achaenia superantes. Achaenia valde obcompressa, nigra, lineari-oblonga, unaquaque facierum circ. 8-striata, faciebus marginibusque adpresse erecto-setosa, non vere alata, corpore 4–5 mm. longa et circ. 1 mm. lata, apice erecte setoso biaristata aristis tenuibus sursum hispidis circ. 1–1.5 mm. longis.

Specimens examined: *Oscar Neumann* 135, alt. 2800 m., mountain meadows, Gardulla, Gallaland, Jan. 14, 1901 (type in Herb. Berl.).

Bidens somaliensis sp. nov.—Herba perennis, erecta, supra erecte ramosa, verisimiliter circ. 9–12 dm. alta, caule glabrato tetragono. Folia opposita, non manifeste petiolata, 8–14 cm. longa et 1.2–3.3 cm. lata, lanceolata vel plus minusve spathulato-lanceolata, inferne sensim ad basim aegre connatam angustata, apice acuta sed non acuminata, acriter serrata plerumque 4–9 dentibus unico latere, subrigida, supra breviter hispida vel glabrata infra dense hispida. Capitula pedunculata pedunculis bracteatis inferne aegre superne valde hispidis circ. 1–1.6 cm. longis, radiata, pansa ad anthesin 4–6.5 cm. lata et 1.2–1.5 cm. alta. Involucri bracteae plus minusve biseriales, exteriores circ. 10–12, lanceolatae vel ovato-oblongae, apice subacutae et induratae, marginibus ac saepe faciebus pilis albis articulatis hispidae, circ. 0.9–1.2 cm. longae, in capitulo juniore disco subaequales; interiores oblongo-ovatae plus minusve hispidae, saepius paulo longiores. Flores radiati 8–14, flavi, ligula subanguste ovato-oblongi, apice minutissime denticulati. Paleae late lineari-oblongae, subrigidae, valde paucistriatae, supra saepe hispidae atque abrupte vel sensim angustatae, demum achaeniorum corpora paulo superantes. Achaenia cuneato-oblanceolata, plana, brunneo-nigra, faciebus sparsim marginibus atque apice dense erecto-setosa, unaquaque facierum nunc circ. 8-nunc circ. 16-sulcata, corpore 1–1.4 cm. longa

et 3–4.5 mm. lata, biaristata aristis acribus brunneo-stramineis, infra medium subsparsim antrorsum setosis supra medium plerumque nudis.

Specimens examined: *Dr. Domenico Riva* 85 (field no. 1306; *Expedition of Eugenio Ruspoli*), in grassy fields and on plains, from Biddum to Volghe, Somalia, Sept. 15, 1893 (2 type sheets in Herb. Florence).

Bidens taitensis sp. nov.—Herba erecta, verisimiliter perennis, glabra vel sparsissime hispida, forsitan 1 m. alta. Folia opposita, principalia petiolata petiolis marginatis ±1.5 cm. longis, petiolo adjecto ±9 cm. longa, circumambitu late deltoideo-ovata, bipinnatisecta, foliolis (lateralibus 2 jugis) membranaceis, subgrosse dentatis, decurrentibus, saepius oblongo-lanceolatis vel ovato-lanceolatis, minute nigro-punctatis, segmentis apicaliter subacutis. Capitula tenuiter pedunculata pedunculis glabratis vel ad summam hispidis, ±1 dm. longis, radiata, pansa ad anthesin probabiliter 3–5 cm. lata et 8–10 mm. alta. Involucri bracteae exteriores circ. 8 vel 9, late oblongae, apice obtusae cartilagineo-indurataeque, marginibus eciliatae, tergo nonnullis lineis nervatae, circ. 8–9 mm. longae et 2–4 mm. latae; interiores late oblongo-lanceolatae dorsaliter subsparsim hispidae, apicaliter dense pubescentes, saepe paulo breviores. Flores ligulati (tantum unicus visus) flavi, ligula lineari-oblongi, nervis pilosi, apice subintegri, ±1.5 cm. longi. Achaenia nunc late cuneato-linearia nunc oblongo-oblanceolata, plana, atra, unaquaque facierum circ. 8-striata, non vere alata, marginibus dense faciebus non nisi supra erecto-setosa, corpore 6–8 mm. longa et 1.5–2.2 mm. lata, apice erecte hispido et concavo vel raro plano exaristata vel brevissime biaristata aristis usque ad 0.5 mm. longis nunc calvis nunc sursum 1–3- hamosis.

Specimens examined: *J. M. Hildebrandt* 2432*a*, alt. 2000–3000 ft., Taita (Teita) Mts., British East Africa, February, 1877 (type in Herb. Berl.).

Hildebrandt's no. 2432 was collected in quantity and proved to be new (*Bidens hildebrandtii*). Specimens were distributed to Kew, London, Berlin, Vienna, and elsewhere. His 2432*a*, however, although collected at the same time and place, seems to have been rare. Apparently only a single specimen was found, and that none too ample. The habit seems nearest that of *Bidens fischeri* (O. Hoffm.) Sherff, of

German East Africa. From that species it differs, however, in being more glabrous, in having the exterior involucral bracts also the achenes much wider, etc.

BIDENS BIPINNATA **biternatoides** var. nov.—Folia pinnata foliolis lateralibus 3–4-jugis, imis tripartitis segmentis lanceolatis, caeteris simplicibus lanceolatisque. Capitula discoidea vel subligulata. Involucri bracteae exteriores superne sensim vel interdum fere subabrupte dilatatae. Achaenia 2-aristata.

Specimens examined: E. O. WOOTON, in cultivated land, Las Cruces, New Mexico, October, 1895 (type in Herb. N.Y. Bot. Gard.).

In its slightly dilated outer involucral bracts this variety makes an approach toward *Bidens pilosa* L. The general aspect of the plant is at once that of the South American *B. subalternans* DC. and of the Old World *B. biternata* (Lour.) Merr. & Sherff, from both of which it differs sharply, however, in its achenes.

Coreopsis negriana sp. nov.—Herba annua, erecta, ±5 dm. alta, caule gracili, glabro vel ad summam subhispido, viridi-stramineo. Folia opposita petiolata petiolis usque ad 12 mm. longis, saepe tenuissimis, petiolo adjecto ±5 cm. longa, 1–2-pinnatisecta, segmentis principalibus lineari-oblongis, membranaceis, supra infraque glabratis, marginibus ciliatis, acriter serratis dentibus interdum in setas elongatas desinentibus. Capitula graciliter pedunculata pedunculis moderate hispidis usque ad 14 cm. longis, radiata, pansa ad anthesin 2–2.5 cm. lata et circ. 6–7 mm. alta. Involucri hispidi bracteae subaequales exteriores circ. 8, lineari-spathulatae, acriter indurato-apiculatae, 3–4 mm. longae; interiores oblongo-ovatae, apicaliter saepe acutae. Flores ligulati circ. 8, flavi, ligula lineari-elliptici, apice obtuso obscure denticulati, circ. 1 cm. longi et circ. 2–3 mm. lati. Paleae nitido-hyalinae, lineari-oblongae vel lineari-lanceolatae, demum conduplicatae ac achaenium amplectentes, maturae circ. 6 mm. longae. Disci florum stigmata subito capilliformi-caudata. Achaenia lineari-oblonga, valde obcompressa, glabra, corpore nigro estriato vel obscurissime multistriato tantum 3–4 mm. longa et circ. 1 mm. lata, marginibus anguste alata alis brunneis glabrisque, apice exaristata ac glabra.

Specimens examined: *Dr. Giovanni Negri* 915 *bis*, alt. 1500 m., Arussi Galla, June 30, 1909 (Herb. Flor., type).

A species close to *C. pachyloma* O. & H., from which it differs, however, in its smaller capitula, also its smaller achenes which are obcompressed, winged, and glabrous. The general habit is deceivingly like that of *Bidens setigera* (*Coreopsis setigera* Schz. Bip.), some of the foliar teeth even having the setiform extensions so notable in that species. But *B. setigera's* achenes are distinctly striate, upwardly setose upon the faces and edges, entirely exalate, and at the apex conspicuously slender-aristate.

CHICAGO NORMAL COLLEGE

EXPLANATION OF PLATES IV, V

PLATE IV

Bidens magnifolia: a, flowering branch, ×0.56; *b*, exterior involucral bract, ×3.36; *c*, interior involucral bract, ×3.36; *d*, ray floret, ×2.8; *e*, palea, ×3.36; *f*, disc floret, ×3.36; *g*, exterior and *h*, interior achenes, ×4.48; *g* and *h*, from *Albers* 204 in Herb. Berl.; rest from *Holst* 2252, type in Herb. Berl.

PLATE V

Bidens cirsioides: a, *b*, lower and upper portions of one flowering branch, ×0.61; *c*, exterior involucral bract, ×3.66; *d*, interior involucral bract, ×3.66; *e*, ray floret, ×2.44; *f*, palea, ×3.66; *g*, disc floret, ×4.88; *h*, achene, ×4.88; all from *Ledermann* 5440, type in Herb. Berl.

SHERFF on COMPOSITAE

SHERFF on COMPOSITAE

SOME EFFECTS OF LOW TEMPERATURES ON SEEDS[1]

W. F. Busse and C. R. Burnham

(WITH THREE FIGURES)

Introduction

It has long been known that air-dry seeds retain their viability after freezing to −190° C. in liquid air (1), but little work has been done to determine whether this treatment has any effect on the later growth of the plant. In experiments carried on in this laboratory, it has been found that many plants, such as maize, wheat, barley, lettuce, onions, and radishes, grow normally after the seeds have been frozen in liquid air. Germination of clover and alfalfa is markedly improved by this treatment (2). With cotton and flax, however, there are some interesting differences between the plants from treated and untreated seeds. This paper is a report of some abnormalities found with these seeds.

I. Cotton

When cotton seeds germinate normally, the hypocotyl grows out of the tip and the cotyledons are gradually pulled out of the seed-coat. Occasionally the cotyledons have difficulty in getting free, but it is nearly always possible in these cases to remove the seed-coat by hand without injuring the cotyledon. If the seeds are cooled with liquid air, either by placing in a container which is then immersed in the liquid air or by placing directly into the air, and are then germinated, the result is different. The percentage of seeds germinating is not changed nor is growth of the hypocotyl greatly affected, but in every case the cotyledons have great difficulty in getting out of the seed-coat. Even when the plant finally succeeds in shedding its coat, the cotyledonary leaves are filled with cracks which look as though the cotyledons had been made brittle while

[1] Contribution from the Department of Physical Chemistry of the University of Wisconsin and the Department of Genetics, Agricultural Experiment Station, University of Wisconsin. Paper no. 111 of the Department of Genetics. Published with the approval of the Director of the Station.

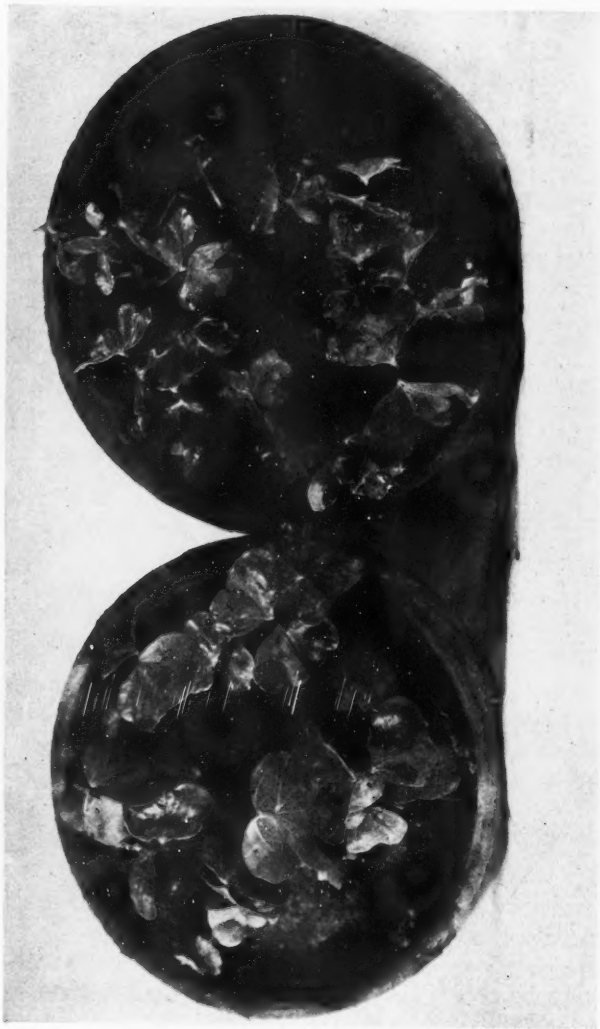

FIG. 1.—Effect of freezing cotton seeds in liquid air: left pot, control; right pot, treated

the seed was at the liquid air temperature. Usually the seed-coat is pushed only part way off, and any effort to remove it by hand results in tearing off part of the already cracked and broken cotyledonary leaves. Fig. 1 shows two lots of cotton plants grown under identical conditions, except that the plants in the second pot were from seeds which had been frozen in liquid air, while the plants in the first pot were controls. It will be noted that the cotyledons of every plant in

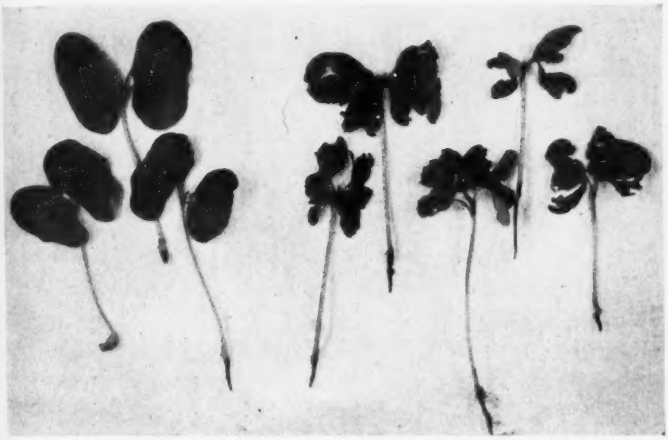

FIG. 2.—Leaves from normal cotton seedlings (left) and from seedlings produced by seeds frozen in liquid air (right).

the second pot are injured, while the controls are normal. Fig. 2 gives a better view of typical leaves from the controls and from plants grown from treated seeds.

The secondary leaves which came out were normal, even though the cotyledonary leaves were badly injured. This seems to indicate that the injury is a purely mechanical one, acting only on the leaves which are already developed. The plants were not grown to a height of more than 25 cm. as the experiments had to be stopped at this stage.

II. Flax

When pure strains of flax seeds were treated with liquid air, it was found that there was little injury to the cotyledonary leaves, al-

though a few of them split along the middle vein. Instead of growing with a single stem in the seedling stage, however, as the control plants did, some of the seedlings from treated seeds grew differently. Nearly all started with a single stem, but this often became split into two separate stems anywhere from two to fifteen nodes (about

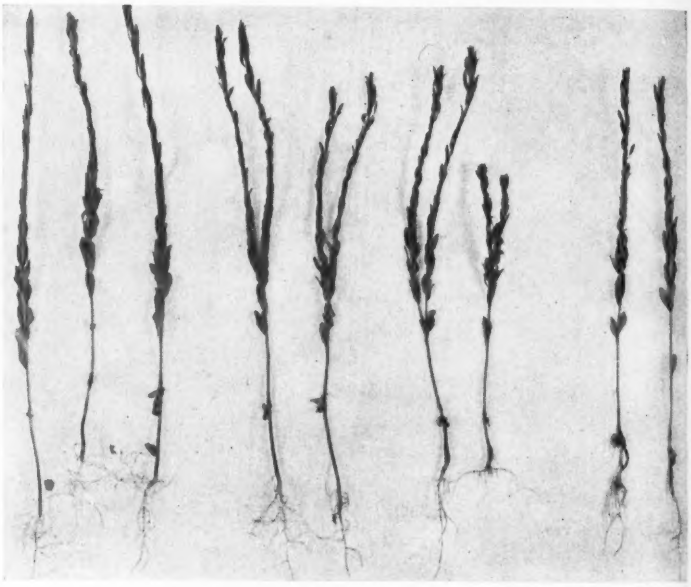

FIG. 3.—Flax seedlings grown from seeds treated with liquid air

1–7 cm.) above the crown of the plant. In even more cases a fasciated and split hypocotyl developed from the seeds treated with liquid air.

Fig. 3 shows the type of plants which developed. The three plants at the left are normal, each having a single stem and a single hypocotyl. The next two plants have twinned stems and single hypocotyls, while the two following have both the stem and the hypocotyl doubled. The last two plants have single stems but double hypocotyls. In one experiment only one plant out of about 20,000 grown from pure lines of untreated seeds showed any fasciation or doubling,

while in some cases freezing in liquid air resulted in more than 70 per cent with these abnormalities. In an attempt to find the factors causing this effect, the following experiments were performed.

Varietal differences and effect of rate of warming on amount of twinning

Ten different strains of flax were used, including Argentine, Indian, Ottawa White-flowered, Abyssinian Yellow-seeded, Crete, and Saginaw selections. These are all seed flaxes except the last, which is a fiber type. To be certain that the strains were pure, lines were used in which natural crossing had been guarded against by bagging individual plants for a number of generations. Two lots of 50 seeds each were treated, and one lot of 100 seeds from each line was used as a control. The treated seeds and controls were planted in alternate rows on a greenhouse bench.

It was noted in preliminary experiments that when the seeds were taken out of the liquid air and immediately placed on a piece of metal to warm up quickly, the mechanical stresses in the seed were great enough to cause it to burst and shatter the outer seed-coat, sending the rest of the seed flying several feet but apparently not injuring it. It was thought that this violent reaction might be associated with twinning of the stems, so two different treatments were given the seeds. Treatment *A* consisted of placing the seeds in liquid air in a Dewar flask and allowing them to remain there until the liquid air had evaporated and the flask and the seeds had warmed up to room temperature, thus giving a very slow warming of the seeds. In treatment *B* the seeds were cooled by placing in liquid air for two or three minutes and then were warmed up as rapidly as possible by taking them out of the liquid air and immediately placing them on a metal plate.

The results of these experiments are shown in table I, which gives the germination, the percentage of plants having double stems, the percentage having a twinned hypocotyl, the percentage having both, and the total percentage having either an abnormal stem or hypocotyl or both. The last three columns give the percentages of plants having extra leaves, such as three or more at a node, or two on one side of the stem at a node, and also include abnormal leaves such as

TABLE I

EFFECT OF FREEZING DIFFERENT STRAINS OF FLAX IN LIQUID AIR

VARIETY	PERCENTAGE GERMINATION			TOTAL PLANTS		PERCENTAGE														
						DOUBLE STEM AND SINGLE HYPOCOTYL			SINGLE STEM AND DOUBLE HYPOCOTYL			DOUBLE STEM AND DOUBLE HYPOCOTYL			TOTAL ABNORMALITIES			IRREGULAR LEAVES		
	Control	A	B	A	B	Control	A	B	Control	A	B	Control	A	B	Control	A	B	Control	A	B
6. Crete	79	68	66	34	33	0	3	6	0	3	6	0	3	0	0	9	12	0	0	6
9. Abyssinian	56	16	28	8	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11. Ottawa	93	90	76	45	38	0	0	4	0	0	0	0	0	0	0	0	4	8	10	10
15. Indian	86	90	94	45	47	0	2	0	0	0	2	0	0	0	0	2	2	10	4	16
16. Argentine	89	86	88	43	44	0	5	2	0	0	0	0	0	0	0	5	2	15	30	22
20. North Dakota	99	98	94	49	47	0	2	0	0	0	0	0	0	0	0	2	0	1	4	10
21. Argentine	98	64	56	32	28	0	13	4	0	16	18	0	9	0	0	38	22	....	....	....
22. Argentine	97	72	62	36	31	0	20	9	0	6	9	0	6	0	0	32	18	....	....	....
22. Argentine	93	51	62	51	62	0	4	8	1(?)	40	8	0	26	37	1(?)	70	52	5	20	46
8A. Common seed	86	86	74	43	37	0	0	2	0	0	2	0	0	0	0	0	4	....	....	....
D5. Saginaw	86	62	70	31	35	0	3	3	0	19	3	0	0	0	0	22	6	....	....	....
8A. Common seed*	67	54	88	27	44	0	0	4	0	0	2	0	0	0	0	0	6	....	....	....
11. Ottawa*	68	58	54	29	27	0	0	4	0	0	0	0	0	0	0	0	4	....	....	....
22. Argentine*	56	30	34	15	17	0	20	12	0	0	12	0	6	0	0	26	24	....	....	....

* Grown in botany department greenhouse; others grown in genetics department greenhouse.

semidouble ones (having two main veins and tips but failing to separate farther back of the tips). The table shows that germination of some of the lots is reduced by the liquid air treatments, but there is no absolute correlation between lowered germination and twinning. There are marked varietal differences in the amount of twinning, suggesting a genetic or heritable difference between strains; but the type of treatment has surprisingly little effect.

The last three samples shown in table I were given the same treatments as the others, but were grown in a different greenhouse to make sure that it was not some peculiar local condition of the soil or atmosphere that was causing the results. While the amount of twinning found here was somewhat less than in the other tests with the same lots of seeds, the results are of the same order in both cases. Only one plant from the 1400 untreated seeds planted as controls in this series showed any suggestion of forming a fasciated or doubled stem or hypocotyl, while in some cases 30–70 per cent of those treated showed double stems or hypocotyls. This would indicate that the phenomenon is due to treatment of the seeds with liquid air.

Effect of time between treatment and planting on amount of abnormalities

Since it is known (3) that even comparatively simple protein systems, such as gelatin solutions, sometimes require days to reach equilibrium after thermal changes, it was thought that the time between thawing of the seed and planting might influence the formation of twinned stems and hypocotyls. To test this, three 50-seed samples of lot 22 were given treatment *B* and one was planted 4 hours later, one 3 days later, and the other 2 weeks later, planting controls with each lot of treated seeds. The results are given in table II.

In the lot of seeds planted 2 weeks after treatment, germination was markedly slower than in controls planted at the same time. Table II shows that the germination percentage was low in all treated lots, but the formation of abnormalities is not greatly affected by storing the seeds two weeks after freezing in liquid air.

Growth of plants following treatment

On the average, plants from treated seeds were not so tall as those from controls, and the abnormal plants were shorter than the normal

ones from treated seeds, as shown in table III. This table gives the heights of plants in the series reported in table II. With the exception of the seeds planted 2 weeks after treating, the amount of variability is much greater in the abnormal plants from the treated

TABLE II

EFFECT OF TIME BETWEEN FREEZING SEEDS AND PLANTING, LOT 22; TREATMENT B

TIME BETWEEN FREEZING AND PLANTING	PERCENTAGE GERMINATION		TOTAL PLANTS TREATED	PERCENTAGE					
				DOUBLE STEM		DOUBLE HYPOCOTYL		TOTAL ABNORMALS	
	Control	Treated		Control	Treated	Control	Treated	Control	Treated
4 hours	93	62	62	0	16	1	45	1	53
3 days	93	67	67	0	23	0	44	0	52
2 weeks	89	57	57	0	30	1	39	1	54

TABLE III

COMPARATIVE HEIGHTS IN CENTIMETERS OF NORMAL AND ABNORMAL PLANTS

TIME BETWEEN FREEZING AND PLANTING	MEASUREMENT	CONTROL	TREATED SEEDS			
			Normal	Single stem, double hypocotyl	Double stem, single hypocotyl	Double stem, double hypocotyl
4 hours	Mean height	17.5±0.25	16.6±0.62	12.9±0.59	13.1±1.23	9.6±0.49
	Standard deviation	3.26	3.94	3.95	4.54	2.87
	Coefficient of variability	18.6±1.06	23.7±2.66	30.6	34.7	29.9
3 days	Mean height	Same as above	14.8±0.52	14.0±0.48	.....	10.7±1.17
	Standard deviation		4.16	3.51	.....	3.93
	Coefficient of variability		28.0	25.1	.....	36.7
2 weeks	Mean height	15.3±0.22	15.3±0.39	12.4±0.48	11.8±0.72	10.9±0.42
	Standard deviation	2.62	3.22	3.08	2.42	1.97
	Coefficient of variability	19.4±1.20	21.0	24.8±2.96	20.5	18.1

lots than in the control. The number of plants having the different types of abnormalities is too small to make it possible to draw any conclusions as to the type of abnormality that is usually associated with the greatest dwarfing.

EFFECT OF MOISTURE CONTENT OF SEEDS

It was thought that the amount of moisture in the seeds when they were frozen might have an effect on the formation of abnormali-

ties, so seeds of different moisture content were treated with liquid air. Two 100-seed samples of each variety were dried 10 hours in a drying room at 51° C., and the moisture content of the other seeds is recorded as the amount above that present in this dry state.

To increase the moisture content, the seeds were placed in an atmosphere of high humidity until the stated amounts of moisture had been taken up and they were then kept in stoppered 2-inch

TABLE IV

EFFECT OF MOISTURE CONTENT ON FORMATION OF ABNORMALITIES FROM FLAX SEEDS TREATED WITH LIQUID AIR

Variety	Moisture	Percentage					
		Germination		Double or fasciated hypocotyl		Double growing point	
		Treated	Control	Treated	Control	Treated	Control
22	0.....	77.3	89.1	68.3	0	15.9	0
	5.....	82.6	90.0	17.6	1.0	6.8	0
	6.....	85.5	.....	39.3	.....	3.4	.....
	7.....	84.5	.....	18.3	.....	7.3	.....
	10.....	89.1	91.3	1.1	0	2.2	0
	15.....	97.3	.....	0	.....	0	.....
11	5.....	84.5	88.2	1.0	0	0	0
	7.....	93.6	.....	0	.....	0	.....
	10.....	92.7	.....	0	.....	0	.....
	15.....	65.5	.....	0	.....	0	.....

test-tubes. The treatment was made by immersing the tubes in liquid air. Two lots of seeds were used, no. 22, which usually gave a high percentage of abnormalities, and no. 11, which gave almost no abnormalities when the seeds were frozen. The results are shown in table IV. These results show that the percentage of abnormal plants of strain no. 22 decreases with increasing moisture content of the seeds up to 10 or 15 per cent moisture. The germination percentages show that liquid air treatment has not killed the seeds of this lot having moisture contents up to 15 per cent. Strain no. 11 showed decreased germination when seeds containing 15 per cent moisture were frozen in liquid air, but there were practically no abnormalities when the seeds of either strain having this moisture content were frozen.

Effect of freezing in CO_2 snow

To determine whether less intense freezing could cause these same abnormalities, samples of strain 22 having various amounts of moisture were placed in 2-inch test-tubes and immersed in a CO_2-ether mixture (−80° C.) for 10 minutes. They were then removed and planted the following day. The results of this treatment are shown in table V. A comparison with table IV shows that CO_2 snow

TABLE V

Effect of freezing in CO_2 snow on seeds of various moisture content

Variety	Moisture	Percentage					
		Germination		Double or fasciated hypocotyl		Double growing point	
		Treated	Control	Treated	Control	Treated	Control
22	0.....	73.6	89.1	68.3	0	15.9	0
	5.....	100.0	90.0	47.0	0	17.6	0
	7.....	90.9	.....	38.8	.....	10.0	.....
	10.....	97.3	91.8	1.3	0	0.0	0

is even more effective than liquid air in causing abnormalities, and like liquid air it is more effective with the drier seeds.

Breeding behavior of plants from treated seeds

Thirty normal plants from treated lots giving abnormalities were grown to maturity in the field; together with five plants having double stems and single hypocotyls and three having double stems and double hypocotyls.

Fifty to one hundred seeds from each of these plants were planted in the greenhouse and observed for the presence of abnormalities. In a total of over 3000 plants, only two with forked stems were noted, and 17 plants with fasciated and two with double hypocotyls were observed. In about 2000 seedlings from control or untreated seeds, five with fasciated hypocotyls were found and none with double stems or hypocotyls. Thus there is no significant difference between the treated and untreated lots. The abnormalities produced by the low temperature treatment are not inherited, but seem to be due to temporary changes in the developmental processes.

Discussion

The results of these experiments raise some interesting questions as to the mechanism by which freezing a dormant seed will, at some later date, cause the growing point of the seedling to develop in two directions, producing doubling of the stem. While it is not possible to give a final answer to these questions, some factors which might be affecting the process may be discussed.

The fact that some pure lines of seeds give a large percentage of abnormal plants when frozen in liquid air or solid CO_2-ether mixture, while other pure lines give few or none when subjected to the same treatments, seems to show that the phenomenon has some genetic basis. These lines have been inbred for so long that the seeds of any one strain should be genetically alike. When they are frozen, however, some seeds of a strain show no apparent effect while others produce abnormal plants. This may be due to variations in external and internal environment and therefore non-genetic. The seed bolls of flax are produced over a long period, and conditions during seed development, such as temperature, moisture, available nutrients, certainly are not the same for all seeds on a plant. In this connection it may be worth noting that plants of nearly all the pure lines show variations in the number of nodes, with two leaves that are produced before the leaves start to grow with just one at a node. Also some of the plants produce extra or double leaves. Unfortunately data were not taken on the double and extra leaves in all cases, but table I gives some evidence that when freezing the seeds causes much twinning of the stem and hypocotyl it also increases the percentage of plants having abnormal leaves. If this is generally true, then exposing the seeds to low temperatures may liberate an inherent tendency of the embryonic cells to separate and form more parts than are usually produced.

Whether this view is true or not, the mechanism by which the change is brought about is probably a physicochemical one. The effect might be due to the simple mechanical stresses and strains set up in the seed owing to different coefficients of expansion and different rates of cooling of the different parts. When these stresses are great enough to burst the seed-coat with a force sufficient to send the seed flying for a distance of several feet, it is evident that the struc-

ture within must be subjected to severe strain, but it is rather difficult to see just how it could affect the cells in the growing point to make them separate and later produce two stems or two hypocotyls.

If the different coefficients of thermal expansion of the different parts of the seed cause what might be called an "inner crack" at the tip of the growing point, this should cause the point to develop into two separate parts. If this is so, it might be possible to produce the same results by very careful micro-probing of the seed. The stresses might also produce a direct mechanical injury to the cells at the tip of the growing point, and when the other cells grow around these, two tips would form.

Another possibility is that freezing alters the protoplasm of the individual cells in such a way that the cells of the growing point fail to respond normally, thus allowing the production of several growing points and hypocotyls as well as abnormal leaves. The fact that most dry seeds are uninjured by freezing makes it easy to lose sight of the fact that freezing might cause injury to the protoplasm in some cases, even when there is not enough water to separate out as ice crystals. MORAN (4) showed that freezing and thawing a gelatin gel caused permanent changes in its volume and in its ability to absorb water. HARDY (3) showed that while normally gelatin gels are singly refractive, part of the gel becomes doubly refractive on freezing and remains so after thawing.

If such effects are observed in this simple system, one must consider the possibility of much more diverse and striking effects in a complex system like protoplasm. It may be that some such change in the protoplasm of the seeds is produced by the freezing, and this causes the formation of twin stems and hypocotyls as well as double and extra leaves. It would be surprising to find flax the only plant thus affected by intense freezing of the seeds, so it should be worth while to see whether other plants would not also produce the same type of effect when the seeds are treated with liquid air. If other such cases were found they might throw more light on the cause of the effect in flax.

Summary

1. Freezing cotton seeds in liquid air injures the cotyledons so that they become cracked and split and are not able to emerge completely

from the seed-coat. The secondary or true leaves do not appear to be injured by treating the seeds with liquid air.

2. When flax seeds of certain strains are treated with liquid air, a large proportion of the plants form double stems and hypocotyls; none of the plants from untreated seeds do this. Other strains rarely if ever produce plants with double stems or hypocotyls when the seeds are cooled in liquid air.

3. The time between freezing the seeds and planting them has little effect on the formation of abnormalities.

4. Increasing the moisture content of the seeds before freezing them reduces the number of plants having abnormal stems and hypocotyls.

5. Freezing the seeds in a CO_2 snow-ether mixture ($-80°$ C.) is somewhat more effective in producing abnormalities than freezing them in liquid air ($-190°$ C.). As with the liquid air, no abnormalities are produced when seeds having about 10–15 per cent moisture are frozen in CO_2 snow.

6. Breeding tests showed that the abnormalities produced are not inherited.

7. The mechanism by which the abnormalities are produced cannot be deduced from the data available, but it seems probable that freezing causes some physicochemical change in the protoplasm of the embryo cells which causes them to react differently to the normal stimuli; or it changes some of the restraining and formative forces, which cause the plant to develop in a more or less fixed way.

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HYDROLYSIS IN THE LIVING PLANT BY POLARIZED LIGHT[1]

ELIZABETH SIDNEY SEMMENS

(WITH NINETEEN FIGURES)

Introduction

During the past five years, a series of simple experiments has been carried out, showing that polarized light has the power of hydrolyzing starch and other reserve products within the leaf of the living plant.

These experiments have been performed in England, Canada, and South Africa, under widely differing conditions of temperature and light intensity, and have given definitely positive and consistent results. Simple as these experiments appear, they nevertheless indicate a fact of great importance in plant metabolism, for all plants, particularly if the sky is clear, are exposed to a constant succession of polarized and non-polarized light.

I. Hydrolysis by polarized light

It is well known that the morning and evening sky is strongly polarized, the maximum polarization lying along a great circle having the sun for its pole, as indicated by testing with a Nicol's prism. It has been found that this light has a decidedly hydrolyzing effect upon starch, glucosides, and other complex organic reserve substances within the leaf, at temperatures considerably below those ordinarily associated with enzyme action.

If a healthy young leaf is exposed to light, polarized either naturally, as in skylight under certain conditions, or artificially by reflection or by transmission through a Nicol's prism, the starch in the mesophyll is found to break down in the area thus exposed; and on staining with iodin a light patch is seen, corresponding in position and shape with this area. There is no doubt that this is due to the orientation of the molecular surfaces. Since plants and animals consist

[1] Paper read at the British Association Meeting in South Africa; July, 1929.

almost entirely of definitely oriented surfaces: starch grains, blood corpuscles, cell walls, membranes, in this selective action of polarized radiation, whether of heat or light, we are dealing with some correlation between the vibration direction of the incident radiation and the orientation of the electron orbit of the molecules at the surface.

The writer's attention has recently been drawn to an investigation[2] which attempted the impossible task of obtaining differential results with polarized light on boiled starch solution, contained in a small vessel, when negative results were obtained. But why should there be any selective effect on the uncontrolled molecules in a test-tube?

The writer, when working in Professor E. C. BALY's laboratory at Liverpool in 1923, also made a series of very careful experiments upon boiled starch. The selective results were extremely small, and it was not felt that they were suitable for publication at the time, but these negative results led to important conclusions and paved the way for further experiments, of which a full account will be given later.

BUNKER and ANDERSON also experimented with starch grains, but as they refer only to unpublished data it is difficult to estimate the value of their results. It may suffice to suggest that, as all their experiments were done at temperatures over 40° C., in the presence of diastase there could scarcely be room for any differential effect.

The writer has always worked at temperatures much below those favorable to enzyme action, and it was stated (British Association Report, 1923) that at 20°–25° C. the selective effect disappeared.

Experimentation

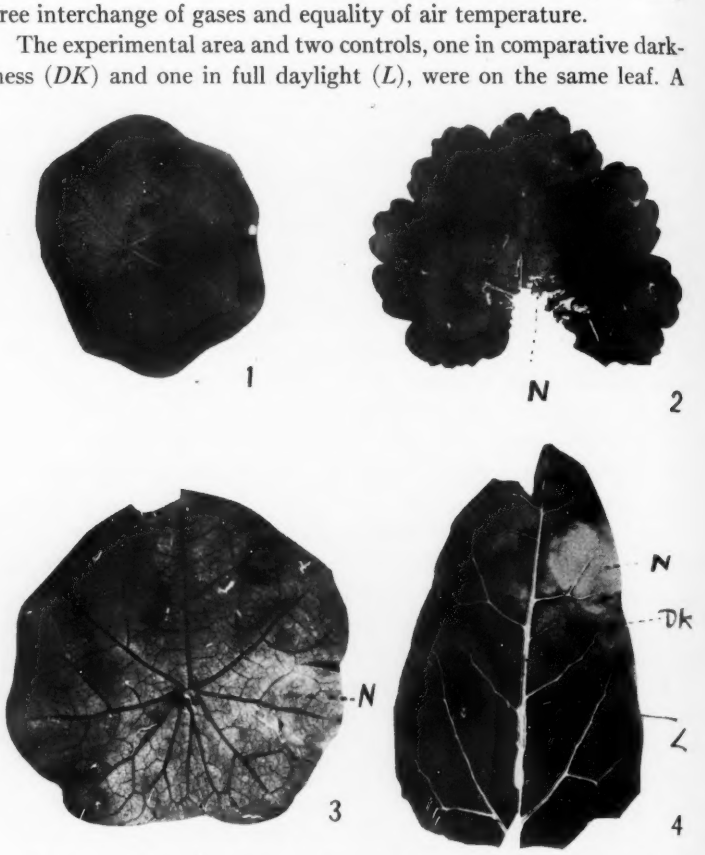
To demonstrate hydrolysis in the leaf, experiments were arranged as follows.

A young and healthy dicotyledonary leaf (preferably of thin texture and without hairs, which have a scattering and depolarizing effect) was exposed to blue skylight, behind a Nicol's prism having a wide rim. The instrument rested lightly against the leaf, which was kept from shifting either by a support to the stem or by a soft cotton pad just touching the back of the leaf. Care was taken to

[2] BUNKER, J., and ANDERSON E., Jour. Biol. Chem. May. 476–487. 1928.

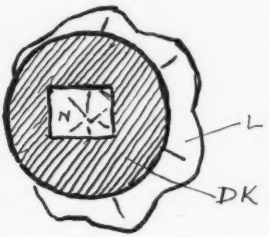
insure that the air was able to circulate freely over the leaf, providing free interchange of gases and equality of air temperature.

The experimental area and two controls, one in comparative darkness (*DK*) and one in full daylight (*L*), were on the same leaf. A



FIGS. 1–4.—Fig. 1, *Tropaeolum* leaf exposed on living plant in E by NE window of cool upper laboratory in Bedford College, London, June 4, 12:30 to 6:30 P.M.; starch remained unchanged in dark but hydrolyzed under polarized light; fig. 2, smooth geranium leaf exposed in cool greenhouse, March 13, London, for 3 hours; starch seen under Nicol's prism; fig. 3, *Tropaeolum*, June 20, College garden, 6 hours; part exposed to daylight is darkened, owing to 6 hours more photosynthesis than *DK*, but part in polarized light is much lighter; fig. 4, spinach leaf, South Africa, College grounds, December 17, 12:45–7:00 P.M., bright hot weather; hydrolysis in *N* and photosynthesis over *L* are very strong (in both figs. 3 and 4 there is probably some hydrolysis in *DK*, owing to stimulation of leaf enzyme by higher day temperature).

careful drawing of the exact position of the Nicol was made and kept with details for reference. After 4–6 hours' exposure, the leaf was picked and one or two pin-holes made to confirm the position of the Nicol. The leaf was then boiled in 95 per cent alcohol, or



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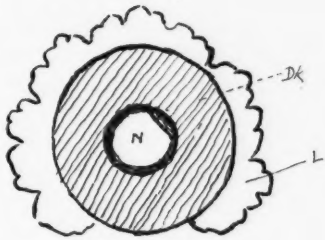

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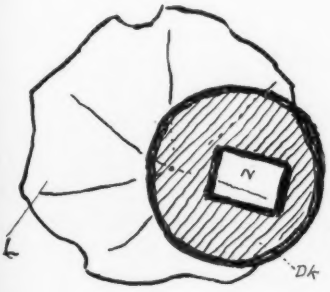

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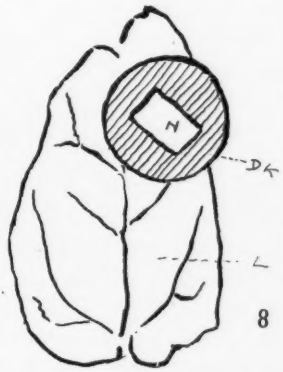

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FIGS. 5–8.—Illustrating shape and position of polarizing Nicol in each case: *N*, area of polarized light behind Nicol aperture; *DK*, area in darkness, behind opaque rim of cork, etc. *L*, area exposed to daylight.

steeped all night and then dipped in iodin in potassium iodide and mounted in glycerin.

As shown in the photographs (figs. 1–4), a light area, exactly defining that exposed to light through the Nicol, was seen. The outer part was very dark, owing to photosynthesis, and the part under the rim in comparative darkness remained unchanged at ordinary

low temperature; but if the weather was warm there was slight diminution of starch, due no doubt to the slow action of the enzyme in the leaf. The rate of starch hydrolysis, therefore, even at comparatively high temperatures, is shown to be many times greater under polarized light than in darkness.

To show that no other factor, such as pressure, temperature, etc., was producing this effect, a further control experiment was made. A leaf was exposed in a similar manner, but the crystal of the Nicol was replaced by a piece of glass. As was expected, instead of the lighter area due to hydrolysis, a dark patch was seen in the center, indicating photosynthesis by the non-polarized light.

HYDROLYSIS OF ANTHOCYANIN.—Observations on young seedlings exposed to polarized light showed a diminution of red coloring matter, and the preceding experiments were therefore repeated with leaves of the beet. A deep crimson leaf of *Beta vulgaris*, of uniform tint, was chosen and over it was placed a Nicol's prism, the back of the leaf resting gently against another leaf and being held lightly in position. After 6 hours' exposure the leaf was picked, plunged into dilute sulphuric acid, and then laid in amyl alcohol for several hours.

Result

At the point of exposure drops of red liquid (anthocyanidin) could be seen dissolving into the amyl alcohol, and after some hours a light patch was seen, taking the shape of the exposed area, indicating that the anthocyanin on the exposed upper surface had hydrolyzed to anthocyanidin and had been dissolved by the amyl alcohol.

In performing these experiments, considerable patience and care are necessary. Too long exposure may result in degradation of the protoplasm and a dark brown spot is seen in the middle of the light area (*N*). The whole tissue may collapse, or the plant be killed by too long exposure. Too short exposure or too weak light may result in the starch changing only to erythro-dextrin, and giving a brownish patch; but if the leaf is washed in hot water and again flooded with iodin solution, this is eliminated and the light patch appears. Great care must be taken in adjusting the Nicol's prism, so as to prevent the oblique non-polarized beam from passing through to the leaf.

The effect can be obtained only with young and healthy leaves.

The leaves chosen for exposure were *Tropaeolum*, *Fuchsia*, *Geranium* (smooth leaf), sow-thistle, spinach, lucerne, and others, all of which gave striking differential results, showing starch hydrolysis under the polarized radiation.

Exposure of a hairy leaf of *Abutilom* gave only a faint outline of the Nicol aperture, showing that the hairs had partly depolarized the light.

Nearly one hundred of these experiments have been made, and a few photographs of results are shown (figs. 1–4).

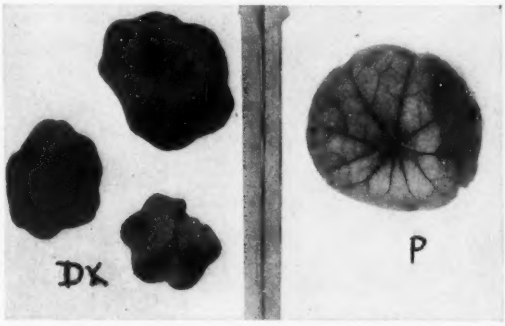


FIG. 9.—Leaves from bed of *Tropaeolum*: *P*, picked at sunset; *DK*, kept in dark and picked at 9:00 A.M. next morning (19 hours).

II. Action of blue skylight

CANADA.—The first experiments were made during a brief stay in Canada, in the month of September.

A bed of *Tropaeolum* was chosen which was lying to the east of a tall building, and thus shielded from the rays of the setting sun but exposed to full sunlight until about 2:00 P.M.

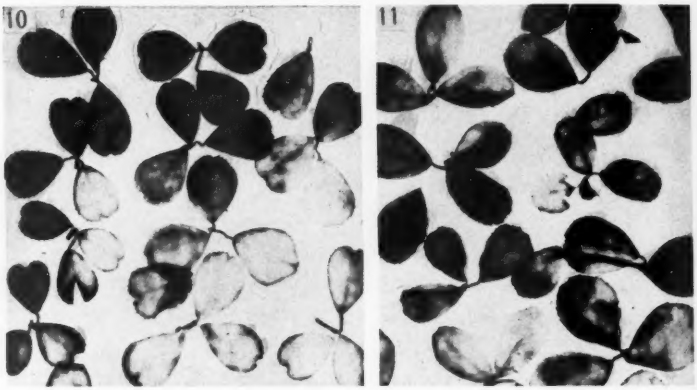
On a cloudless day a small spray of young leaves was covered at this hour and left till 9:00 A.M. the next morning, when it was picked and plunged into 95 per cent alcohol. A young adjacent leaf, having the same aspect, was noted at the same time but picked at 6:00 P.M. on the first evening, having thus been exposed for 4 hours to the polarized afternoon skylight.

On staining with iodin, this second leaf (fig. 9 *P*) was found to be almost denuded of starch, while the leaves (fig. 9 *DK*) which had

been in darkness for 19 hours had full starch content. The experiments were carried out in comparatively cold weather, but after a day of torrential rain, followed by a slight frost, no further reliable results could be obtained.

ENGLAND.—The investigation was discontinued for some time, but when working in England upon the effect of light polarized by a Nicol on the leaves of sow-thistle, attention was again directed to it.

A leaf shaded from the setting sun, picked in the evening of a cloudless day, gave very little starch, but when a bright morning



FIGS. 10, 11.—Leaves from patches of lucerne: fig. 10, exposed to afternoon polarized light; fig. 11, from adjoining patch kept in darkness.

was followed by a dull afternoon with slight rain in the early evening, in a similarly situated leaf the full starch content remained. This could only be explained by concluding that in the first case the starch had been hydrolyzed by the clear blue skylight (which on testing was found to be strongly polarized), while in the second the light was depolarized by cloud and moisture, and thus no hydrolysis took place.

SOUTH AFRICA.—In carrying out the experiments upon spinach leaves already described, it was noted that when the leaf was placed in such a position as to receive skylight from the area of polarization, but was shaded from the sun, that part of the leaf exposed to daylight had less starch than the part under the wide rim of the

Nicol's prism, which was in darkness. As a consequence of these preliminary results, series of investigations were undertaken and photographs of some of the results are given (figs 10–15).

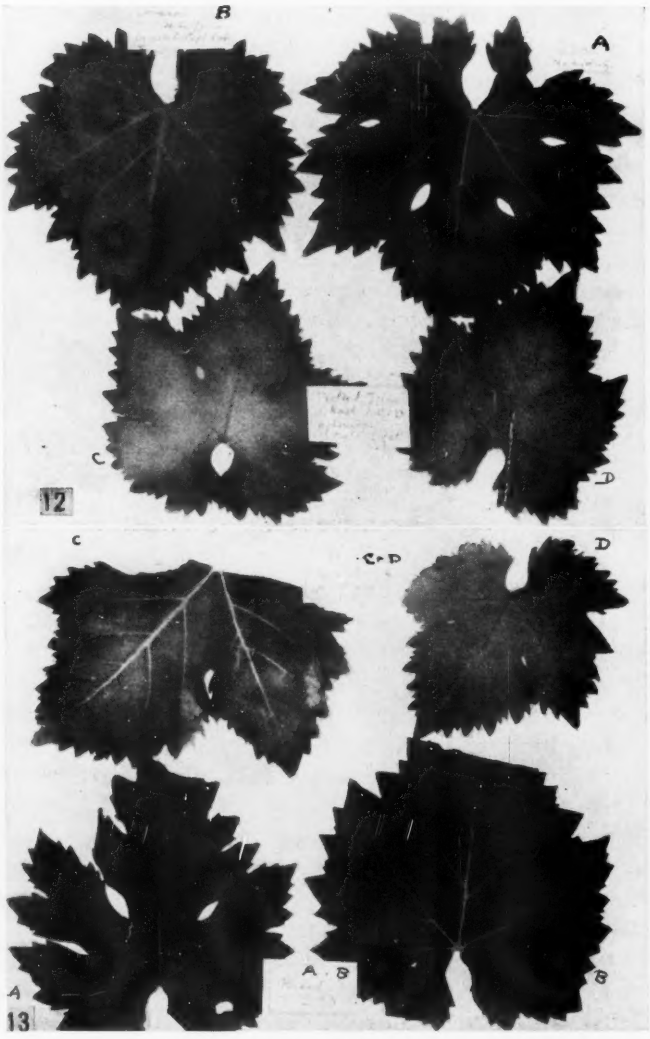
First series

Small patches of young lucerne plants, growing on the ground and of the same age and appearance, were chosen, so that one (fig. 10 *P*) could be exposed to the afternoon skylight in that part of the sky having maximum polarization, but shielded from the sunlight, while the other (fig. 11 *DK*) was lightly covered by a thin wooden box, giving ample admission of air. The temperature was noted under such conditions to be exactly the same in the two cases. The experiment was started at 4:00 P.M., and the two sets of leaves were picked at 6:00 P.M., choosing those leaves which stood out best to receive both the mid-day light and in one case the afternoon light. The experiment was repeated several times with similar effects. On one occasion the sky became cloudy and then no difference was observed, as the light was only feebly polarized.

Second series

Most interesting results were obtained with vine leaves. In the College grounds are two rows of stone pillars, about 2 feet square, round which large vines are trained. Leaves were chosen growing on the east side of these pillars, these being shielded from the afternoon sun after 1:00 P.M. but pointing to that part of the sky at which the average polarization was greatest. All the leaves received full sunlight up till 1:00 P.M.

Experiment I (fig. 12).—Four of the freshest leaves were chosen: *A* was picked at 4:00 P.M., having received only morning and early afternoon light; *B* was partly covered by a thin dark envelope, leaving the lobe *P* exposed; *C* and *D* were left fully exposed to the blue polarized skylight till sunset at 7:00 P.M. On staining with iodin *A*, which had 3 hours less daylight than *C* and *D*, had full starch while the latter showed faint purple only at the edge. The partly covered leaf showed less starch in the exposed part than in the part in darkness. (The line of demarcation is not sharp, because the envelope was only lightly attached, to give free play of air to the surface of the leaf.)



FIGS. 12, 13.—Fig. 12, vine leaves: *A*, picked 4:00 P.M. after bright morning; *B*, right leaf half-covered, lobe *P* being left exposed 4:00–7:00 P.M.; *C*, *D*, picked 7:00 P.M., having had 3 hours more bright skylight than *A;* fig. 13, *A*, *B*, picked at 2:30 P.M.; *C*, *D*, picked at 7:00 P.M., having had 4½ hours more skylight than *A* and *B*.

Experiment II (fig. 13).—In this case *A* and *B* were picked at 2:30 P.M. and showed full starch content; *C* and *D* were left till sunset and were almost devoid of starch. (*C* was a large leaf and there was only room for part of it. A large leaf was chosen together with a small one, to show that size was not the determining factor.)

THIRD SERIES

Experiments upon *Tropaeolum* were equally interesting. Two pots of young plants of the same age and size were taken. One was lightly covered from 4:00 to 6:00 P.M., the other being exposed to polarized skylight (*P*). The most prominent leaves were picked and treated with alcohol and iodin.

The results are seen in figs. 14 and 15. Again the polarized light of the sky caused hydrolysis, while in darkness the starch remained practically unchanged.

Result

The invariable result of the numerous experiments performed, only a few of which are here reported, must lead to the conclusion that starch, which is formed in the sunlight and non-polarized daylight, is digested by the polarized light of the late afternoon (and early morning), especially when the sky is clear. In cloudy weather it is probable that the chief element is the enzyme of the leaf, which acts slowly in the dark and is dependent on temperature. The remarkable acceleration of the process of digestion produced by polarized skylight must greatly increase the rate of growth of the plant. However plentiful the food supply, a living organism can only use that which it digests.

III. Effect upon growth of whole plant

It is evident that the hydrolysis resulting from polarized light must have an important bearing on the growth of the whole plant. One or two simple experiments have been made in this direction, which may be of interest in paving the way for further systematic investigation.

As a simple test, a young plant was placed on a laboratory bench so that it received only light polarized by a polished reflecting surface, the direct light from the window being excluded. Although sufficiently watered, the plant died in about a fortnight. As an in-

FIGS. 14, 15.—Fig. 14, *Tropaeolum*, exposed to polarized skylight 4:00–6:00 P.M.; fig. 15, from adjoining pot, covered 4:00–6:00 P.M.

quiry into the cause of such death the following experiment was arranged.

A long box, divided into separate compartments, was placed on its long side in front of a north window (N by NE). The backs of these compartments were formed by sheets of wood tilted at an angle of 53° with the horizon. The back of the polarizing chamber was covered by ferrotype, upon which was a sheet of glass. The light from the window was thus polarized by the polished surface and reflected vertically downward upon the plant within the chamber. The back of the control chamber was covered with a piece of dull rough paper, which could be changed to increase or diminish the brightness of this diffused non-polarized light. The direct light from the window was intercepted by a screen in front of the lower half of the two chambers. Plant *P*, therefore, in the polarizing chamber, received a diurnal succession of polarized light and darkness, while plant *L* in the control chamber experienced ordinary light, alternated with darkness. Intensity of the light was tested by photographic paper, placed in similar positions within the two chambers, and was found to remain remarkably equal.

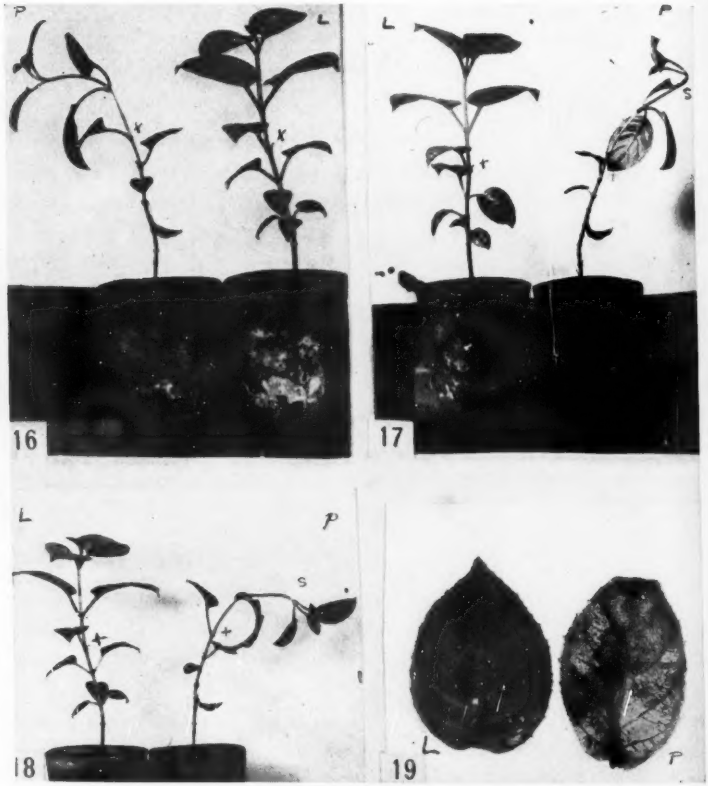
Equal amounts of water measured with a pipette were given to each plant daily.

Result

Both plants, which were chosen by an expert as being similar in height, number of leaves, and healthy appearance, remained in full vigor during the first two days. *P* looked somewhat more vigorous, as its digestion was being wonderfully improved. Soon it began to weaken and show signs of starvation, however, and the extraordinary contortions shown in figs. 16–18 were produced by the hydrolyzed products of the leaf passing down one side of the stem, causing increased turgor on that side. This swelling can be seen on the stem at *S*. The plant was placed so that the polarized light fell full on one or two leaves and the stem turned away from the polarized light. When the pot was turned round so that the inclination was now toward the polarizing surface, the plant persistently turned away again. This occurred most strongly at midday, when the light was brightest. The deflection at one time amounted to about 90° in 1.5

ered 4:00–6:00 P.M.

hours. In the darkness it recovered, and in the early morning the stem was found again to be erect.



FIGS. 16–19.—Figs. 16–18, contortions of stem of young fuchsias, owing to passage of products of hydrolysis down the stem; in each case plant turned away from direction of polarized light; fig. 19, *P*, disappearance of starch and attenuation of leaf tissue of plant exposed in polarizing chamber; *L*, from control in ordinary diffused light, here starch remained and leaf grew healthily.

A leaf was picked from each plant, the position being similar (marked +), and tested for starch. With iodin the control gave a clear purple color, while *P* (fig. 19) was not only practically devoid of starch, but was thin and emaciated.

This corresponds with the fact noted in part I, that where long exposure of the leaf was made behind a Nicol's prism the leaf tissue itself broke down. The experiment was continued for several weeks, and gradually the leaves on *P* dropped off, leaving only two at the apex. The plant was found bending completely over to the ground, but on taking it out of the polarizing chamber and placing it near the laboratory window in ordinary diffused skylight, it recovered its erect posture and began to show signs of returning health.

The control maintained throughout a steady growth and an almost erect position (figs 16–18). Both plants contained an amount of anthocyanin in the young stems. This disappeared in plant *P* but remained in the control. This fact is of interest when compared with the result described in part I, where the anthocyanin in a leaf of *Beta vulgaris* was hydrolyzed to anthocyanidin by exposure for several hours behind a Nicol's prism. This same absence of anthocyanin was observed in another experiment, where mustard seedlings were grown under polarized light, although it was present in the control in ordinary light.

Summary

Plants grown in polarized light alone, succeeded by periods of darkness, exhibit the following results: (1) disappearance of starch and other reserve products, such as glucosides, etc.; (2) temporary phototropism (usually negative, so-called) owing to increased turgor in stem; (3) leaf fall and signs of starvation in whole plant. The second of these effects will, of course, depend on the morphology of the stem and leaf.

The inference is evident, and is in full accord with the interesting observations upon the growth of seedlings by MACHT.[3] For rapid and healthy growth the plant requires the alternation of polarized and non-polarized light, such as is provided by nature on a bright summer day.

I am greatly indebted to the authorities of Bedford College, London University and of the Huguenot University College, South Africa, for granting me facilities for this research. I heartily thank

[3] MACHT, D. I., Influence of polarized light on the growth of seedlings. Jour. Gen. Phys. 1:41–52. 1925.

President BERTHA STONEMAN for her valuable advice and assistance in the investigation. I am also indebted to Dr. FRANK THONE. I should like also to tender thanks to Mr. F. T. LACEY, and Miss WILLIAMS of the Chelsea Polytechnic, and also Mr. HALES of the Chelsea Physic Garden for much help and provision of facilities in the early stages of this work. Lastly, I express great appreciation of the help given me by the late Dr. E. E. SLOSSON.

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BARK STRUCTURE OF CALLIXYLON[1]

CHESTER A. ARNOLD

(WITH SIX FIGURES)

Some details of bark structure are shown in a few specimens of *Callixylon* wood recently collected from the Genundewa limestone horizon at Bristol Center in Ontario County, New York. Although not well preserved and fragmentary, these specimens are of considerable interest because of our scant knowledge of the phloem and cortex of Devonian plants in general. This lack of knowledge is especially true of the phloem and the inner portion of the cortex. The outer cortex, which frequently contains strands of sclerenchymatous fibers, is often found in connection with petrified stems, but usually all structures between this and the wood have completely disappeared.

Several years ago PENHALLOW[2] described and figured the bark of a specimen from about the same locality and horizon as the material dealt with in this account. This specimen, which he described as *Cordaites hamiltonense*, was so poorly preserved that an accurate description of the wood was not made. His figures of the bark structure correspond closely to the material described in this account, and because of the abundance of *Callixylon* wood and the almost complete absence of other types in that region of New York, it is probable that he was working with this form also.

In this account the term "bark" is used as an inclusive designation of all the tissues concerned, since it is impossible to distinguish clearly the cortex from the phloem in the material at hand. Because of its proximity to the xylem, the inner portion of the bark at least is certainly phloem, but the slight differentiation of the tissues farther out is not sufficient to mark a boundary between the phloem and the cortex. Moreover, it is barely possible that the entire structure is phloem, the cortex not being present.

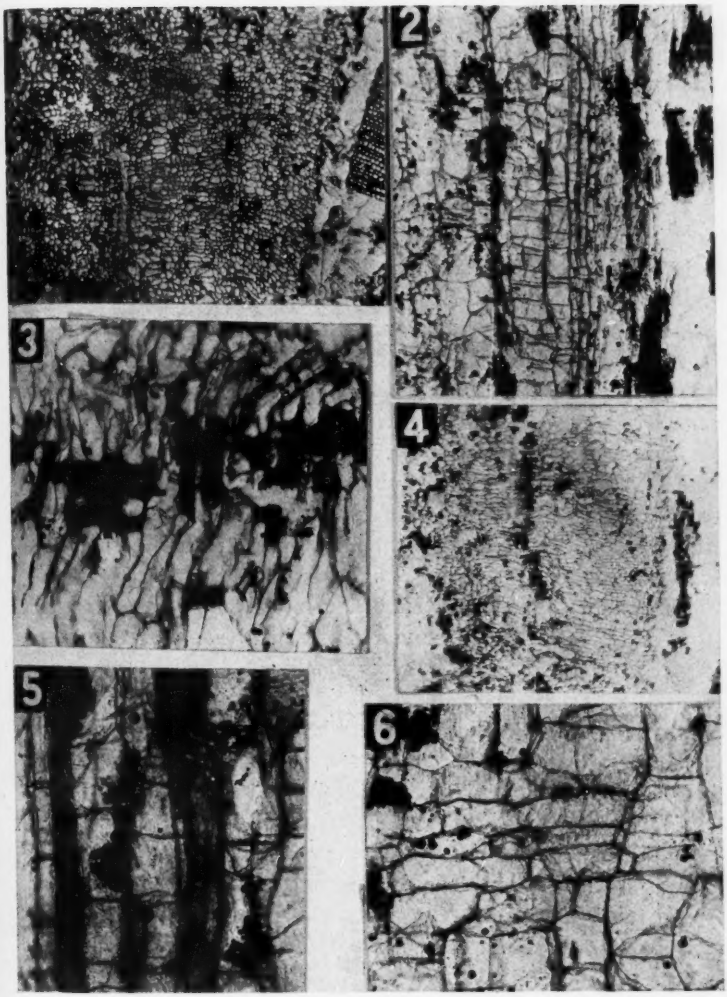
[1] Paper from the Department of Botany of the University of Michigan, no. 327.

[2] PENHALLOW, D. P., Notes on the North American species of *Dadoxylon*. Trans. Roy. Soc. Canada 6 (4):51–77. 1900.

This study was made entirely from radial and tangential sections. There was sufficient material for several sections, but all the tangential cuts were through portions too poorly preserved to be studied satisfactorily. In all instances the amount of bark preserved was small. The material consists of the woody axis of a root 9 mm. in diameter, bearing a bark fragment 3 mm. thick, and a stem 3.5 cm. in diameter, embraced in about one-fourth of its circumference by a bark layer 2 mm. thick. In the latter specimen the bark and wood were separated by an empty space about 3 mm. wide. That this space was caused by a forcing apart of the wood and bark during crystallization of the petrifying agent and not by decay of intervening tissues is indicated by fragments of phloem still in contact with the wood, the splitting having taken place in some instances a short distance from the cambium.

The preserved portion of the bark of the root, in addition to being thicker than that of the stem, shows some structural differences probably associated with the age of the specimens. Adjacent to the xylem is a thin, partially crushed layer of radially arranged cells (not shown in illustration) which was beyond doubt the functional phloem. Just outside this, where preservation is better, the tissue is made up largely of thin walled empty cells, and scattered throughout are clusters made up of few to twenty or thirty cells with a dark content which was probably originally tannin (fig. 1). In cross-section the dark cells are nearly square and measure about 30 μ in diameter. The empty cells are in two general types: some of them are elongated radially and others have their transverse and radial diameters about equal. In the outer portion of the bark they become more irregular in size and arrangement, the radial elongation becomes less apparent, and some of them become of greater diameter tangentially than radially.

In the stem, which was older than the root, a slightly different arrangement is apparent. The tissue of empty cells alternates in a radial direction with layers of tannin cells, the latter being two to five cells in radial extent (fig. 4). The continuity of these bands is frequently interrupted by small groups of empty cells (fig. 3). Throughout the empty celled tissue between these bands of tannin cells are a few scattered tannin cells, either occurring singly or in



FIGS. 1–6.—Fig. 1, transverse section through bark of *Callixylon* roots showing scattered groups of tannin cells, ×17; fig. 2, radial section through bark of stem, ×35; fig. 3, portion from near center of fig. 4 but more highly magnified, ×70; fig. 4, transverse section through bark of stem showing two types of empty cells and banded arrangement of tannin cells as contrasted with scattered arrangement in root, ×17; fig. 5, portion of radial section of phloem similar to fig. 2 but more highly magnified, showing two types of empty cells and their relation to tannin cells, ×115; fig. 6, portion of phloem ray, ×115.

groups of two or three (figs. 3, 4). The tannin cells, when viewed longitudinally, are several times as long as broad and appear to have oblique end walls. As in the root, there are two types of empty cells: those with radial and vertical diameters about equal but shortened tangentially, and others having radial and shortest tangential diameters about equal but exceeded three or four times by the length (figs. 2–5). The latter type of cell seems to occur mostly in close proximity to the tannin cells. Other thin walled cells in which the greatest dimension is radial are so arranged as to stimulate phloem rays (fig. 6). In transverse section these rays are not readily distinguishable but they are conspicuous in radial section.

Structures resembling sieve plates are not distinguished with certainty, and all the cells except possibly the tannin cells have transverse end walls. As already stated, it is uncertain whether this tissue is phloem or cortex except for its proximity to the xylem and for the structures which appear to be phloem rays. It is possible that a considerable quantity of it is old phloem which has undergone alteration, but the younger and functional phloem is small in amount and not well preserved.

Unlike the secondary xylem of *Callixylon* the phloem (assuming that it is phloem) shows comparatively little differentiation. Some of the inner poorly preserved portions show a radial arrangement indicative of cambial origin, so this form scarcely supports the contention of some investigators that the early vascular plants were without true phloem. The scarcity of phloem in vascular plants is generally due to its destructible nature and its location on the outside of the wood axis where it is subject to various destructive forces.

As stated, PENHALLOW'S *Cordaites hamiltonense* (which is probably *Callixylon*) shows bark structure similar to that just described. He says that the "resin canals" (tannin cells) are devoid of transverse septa, but he probably failed to observe them as they are obscure and difficult to see.

In *Mesoxylon sutcliffii*, a cordaitean species whose phloem is described by MASLEN,[3] a more complex organization exists, a distinct pericyclic zone separating phloem from cortex. The cortex shows

[3] MASLEN, A. J., The structure of *Mesoxylon sutcliffii* Scott. Ann. Botany **25**:381–412. 1911.

pronounced periderm formation which, if it existed in *Callixylon*, is not preserved. The phloem contains vertically elongated cells with dark contents apparently very similar to those in *Callixylon*, but the rays and the radial arrangement of the elements are more pronounced.

Since *Callixylon* is Upper Devonian and *Mesoxylon* is from the Lower Coal Measures it is especially interesting to find the simplest bark structure in the older form. The same cannot be said of the secondary wood. Although *Callixylon* is one of the oldest known members of the cordaitean complex, the radial arrangement of the pit groups in the wood appears to be a highly specialized feature. Also there is an enormous development of secondary wood, as shown by the recent discovery of trunks 3 feet in diameter and several feet in length. The cryptogamic primary wood, which is similar to that of *Pitys*, probably places it below the true cordaiteans in the evolutionary scale. The discovery, then, of the rather simple phloem and cortex, although fragmentary and small in amount, suggests that *Callixylon* was a rather primitive plant in which development of the secondary wood was probably out of proportion to that of the other tissues.

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CURRENT LITERATURE

BOOK REVIEWS

Recent investigations of Devonian plants

A valuable contribution to our knowledge of American Devonian plant structures is a doctor's thesis[1] which has been prepared at Cornell University. The material was obtained from a region in the west central portion of the state of New York, primarily from the Genundewa limestone, but also from the Genesee shale which underlies the Genundewa limestone, and from the Portage group, which is above the limestone. The three horizons belong to the Upper Devonian. The preservation in which the plant material appeared is that of wood fossilized by saturation with calcium carbonate; also preservation by iron sulphide (either pyrite or marcasite) occurs. This fossil wood belongs to the genus *Callixylon*, which was founded by ZALESSKY in 1911 on some material from the Upper Devonian of the Donetz basin, but later was found in the Devonian of Ohio and described by DAWSON, PENHALLOW, ELKINS and WIELAND, and HYLANDER.

ARNOLD made thin sections from twenty-four different specimens, seventeen of which are mentioned in his paper. He establishes four new species. *Callixylon zalesskyi*, *C. mentethense*, *C. erianum*, and *C. bristolense*. ARNOLD shows that *Callixylon* was one of the dominant types of the Upper Devonian period. It had already a high degree of specialization, and cannot be considered as primitive in spite of its mesarch primary wood. It distinguishes itself by its grouped pitting from Cordaites, which had continuous pitting. *Callixylon* is characterized by the absence of leaf gaps, and it appears that this genus represents the termination of a very ancient group of plants from which Pteropsida developed at a very early period. The occurrence of scattered medullary tracheids in *Callixylon* would suggest its origin from a previous protostelic complex. *Callixylon* must have been a highly organized plant which probably flourished during the Upper Devonian to the same extent that Cordaites did during the Carboniferous. It was probably not in the line of direct descent of later forms, but reached its climax and disappeared at the end of the Devonian or early Mississippian. It probably sprang from a stock common to both the Pteridospermeae and the Cordaitales.

ARNOLD'S valuable investigation supports SCOTT'S statement that *Callixylon* is the most highly differentiated wood structure, and is also the oldest member of

[1] ARNOLD, C. A., The genus *Callixylon* from the Upper Devonian of central and western New York. pp. 50. *pls.* 19. 1 map. Reprinted from Papers Michigan Acad. Sci., Arts & Letters. Vol. XI. 1929. Published 1930.

the Pityeae. It is a diversified genus, terminating a pronounced evolutionary process which was either gradual and long or intense over a shorter period. Whether it was pteridospermous or cordaitean or something different is a question to be settled only on the acquisition of more information.

To the series of contributions to the study of the Devonian floras by KRÄUSEL and WEYLAND[2] has been added a third instalment. The third number of the series contains a description of the following species: *Asteroxylon elberfeldense* Kräusel and Weyland, *Aneurophyton germanicum* K & W, *Hyenia elegans* K & W, *Calamophyton primaevum* K & W, *Cladoxylon scoparium* K & W, *Protolepidodendron scharyanum* Pot. & Bern., *Haspia devonica* K & W, *Kicklingia erecta* K & W; and a new genus, *Duisbergia* K & W. A number of spores and sporangia and several types of wood showing structure are also discussed.

The authors claim that the Devonian plants are a key for a morphogenetic understanding of the higher plants; they represent types from which all the others can be derived. They assume that these Devonian plants represent the oldest known vascular plants, but those plants already are very far removed from the age of thallophytes.

This number of the series contains 43 pages of quarto and 15 plates. It is to be hoped that the authors will continue with these valuable contributions to our knowledge of the Devonian floras.—A. C. NOÉ.

Physiology and biochemistry of bacteria

The second and third volumes of BUCHANAN and FULMER'S[3] monumental work on the physiology and biochemistry of the bacteria have been published. The first volume of this treatise was noted[4] two years ago. The chapters in the three volumes are numbered consecutively, although the volumes are each paged separately. Volume II covers the effects of environment on organisms, and contains chapters vi–xiii, while volume III, the effects of microorganisms on the environment, contains chapters xiv–xix. This last volume is particularly valuable to students of anaerobic respiration and fermentations.

The discussion is organized into five sections, A, B, and C in volume II, and D and E (mislabeled A and B) in volume III. Section A presents the problems of recognition and measurement of the effects of environment on microorganisms. Section B discusses the effects of physical factors in the environment on bacterial and other microbial life. Section C deals with the effects of chemical factors of the environment. Section D considers special physiological interrelations of mi-

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[2] KRÄUSEL, R., and WEYLAND, H., Beiträge zur Kenntnis der Devonflora. Frankfurt. Senckenbergische Naturforschende Gesellschaft. I. 1923. II. 1926. III. 1929.

[3] BUCHANAN, R. E., and FULMER, E. I., Physiology and biochemistry of bacteria. Vol. II. Effects of environment upon microorganisms. 8vo. pp. xviii+709. Vol. III. Effects of microorganisms upon environment. Fermentative and other changes produced. 8vo. pp. xvi+575. Williams and Wilkins. Baltimore. 1930.

[4] BOT. GAZ. **86**: 118. 1928.

croorganisms, particularly antagonisms, commensalism, and symbiotic relationships of many kinds. The first chapter in the final section, E, deals with the chemical agents of decomposition, the enzymes, including hydrolases and anhydrases, esterases, deaminases, reducing and oxidizing enzymes, etc. This is an important chapter to students of general physiology. There follows a general discussion of chemical changes produced by microorganisms in their environment, and then a more specific discussion of the changes produced in inorganic (carbonless) compounds; changes in the non-nitrogenous organic compounds; and in the nitrogenous constituents of the environment.

In each volume the literature lists and indices occupy 150–170 pages. The three volumes together total 1800 pages. It is difficult to overstate the value of such a work. It is a great storehouse of information, bringing together an immense and inclusive literature which is inaccessible to the ordinary student because it is so widely scattered, and because the process of locating and consulting the original works is so time-consuming.

The authors deserve great praise for their willingness to perform such a service to the science of bacteriology and physiology. In some places the material does not seem to be as well digested as it should be, but it is an extremely difficult task to evaluate the contributions over so vast a field, and draw them together into a well balanced, critical review. The work will prove extremely useful, and should stimulate work by bringing into relief conflicting views of bacterial physiology. It ought to meet the approval and appreciation of a large group of scientists and scientific organizations.—C. A. SHULL.

Inheritance in fungi

A timely and welcome contribution to genetic literature has been supplied by KNIEP.[5] A competent summary of the genetic data relative to the fungi has been needed ever since it was demonstrated for some fungi that they, and therefore all fungi, probably do not stand apart from other plants in their mechanism of inheritance. The observations that in many fungi there is an extensive haplophase which can be cultured and observed as an independent organism, that heterozygosis occurs in many cases and that it can be experimentally manipulated in some, provide instruments for direct demonstration of segregation of genes. The author restricts himself to a discussion of those instances of variability of fungi in which characters are involved that are manifest in the genotype and are passed through the zygote to the progeny, thus eliminating most of the cases which have been interpreted and reported as mutations.

The subject matter is subsumed under (A) chromosome inheritance, which constitutes the major part of the discussion; and (B) plasmatic inheritance, a brief discussion which interprets GOLDSCHMIDT'S studies on *Ustilago violacea* and HARDER'S merogony experiments with *Pholiota mutabilis* as giving a basis for assuming plasmatic inheritance in fungi. The major portion of the discussion of

[5] KNIEP, H., Vererbungserscheinungen bei Pilzen. Bibliographia Genetica V. pp. 371–478. *figs. 15.* Martinus Nijhoff. Gravehage. 1929.

chromosome inheritance is devoted to the phenomena of sex inheritance, and the remainder to inheritance of vegetative characters.

There is a comprehensive bibliography and a detailed index which add greatly to the usefulness of the volume.

The presentation is clear and critical. Its intelligibility is enhanced by illustrations, diagrams, and tables, many of which are original. The reader will find the free use of well chosen illustrations a great aid in the discussion of DODGE'S interesting studies of the genetics of *Neurospora sitophila*.

The volume is marked by the same mastery of facts and fertility of ideas which characterized the author's preceding volume, *Die Sexualität der niederen Pflanzen*.—G. K. K. LINK.

Elementary course in general physiology

A textbook and laboratory manual combined in a single volume has been prepared by SCARTH and LLOYD[6] of McGill University. In order to bring out the similarities and differences between cells and physical and chemical systems, the chapters are arranged more or less in pairs, as indicated by the following chapter headings: Life as a mechanism; Organization of protoplasm; Cell structure; Surface tension in physical systems; Surface tension in cells; Adsorption in physical systems; Adsorption in cells; Diffusion and osmosis in physical systems; Diffusion and osmosis in cells; Ions and their determination in physical systems; Ions, particularly H-ions, and their determination in cells; Electric potential and electric currents in physical (non-metallic) systems; Electric potential and electric currents in cells; Colloids in physical systems; Colloids in the cell. These chapters occupy 190 pages of text. Part I deals with principles and theory; part II presents a laboratory outline, running more or less parallel to the text material. The last two outlines deal with ultramicroscopy and enzymes.

The book will prove suggestive to many who have wanted to bring into the laboratory work in biology something of the flavor and technique of physical chemistry and physics. It is not possible to include everything one might desire in so small a space, of course, but the authors have illuminated many of the processes of living cells for the student who is making his first attempts at understanding the processes of organized living beings.—C. A. SHULL.

Number 9 of volume I of *Die Pilze Mitteleuropas*[7] has appeared. It is a continuation of the Boletaceae, and presents *Boletus parasiticus* and *B. lignicola* in two colored plates. In addition, *B. sulphureus* is illustrated in color in one of these plates. Plates in black and white illustrate *B. luteus*, *B. bovinus*, *B. variegatus*, *B. parasiticus*, and *B. lignicola*. The text is devoted to *B. parasiticus* and *B. lignicola*.—G. K. K. LINK.

[6] SCARTH, G. W., and LLOYD, F. E., Elementary course in general physiology. 8vo. pp. xxii+258. John Wiley and Sons. New York. 1930.

[7] Die Pilze Mitteleuropas, under the editorship of KNIEP, H. (Berlin); CLAUSSEN, P. (Marburg); and BASZ, J. (Stuttgart). W. Klinghardt. Leipzig. 1928.

Microbiology

A volume on microbiology by LUTMAN[8] differs from other textbooks which cover essentially the same subject matter, that is, the morphology and physiology of bacteria and fungi and the significance of these in the economy of nature and in the lives of humans. Unlike textbooks in bacteriology in which usually only passing attention is given to the fungi (yeasts and "molds"), and textbooks in mycology in which no or the merest mention is made of bacteria, this volume treats both of these assemblages of organisms so that a sense of their relatedness and equal importance is gained, even though more chapters and pages are devoted to bacteria than to fungi. This is accomplished by organization of the general physiological aspects of microorganisms about types which include such fungi as *Rhizopus nigricans* and other phycomycetous allies, and *Saccharomyces* sp., *Penicillium* sp., *Aspergillus* sp., together with such ascomycetous allies as *Sclerotinia cinerea*, the rotter of fruits in the orchard, transit, storage. In these chapters the phenomena of growth and invasion of other organisms are discussed in their relationship to temperature, humidity, and soil factors, as well as the phenomena of digestion and respiration in microorganisms. Naturally fermentation receives considerable attention.

The author has found it necessary to deviate from the type method of presentation, and to use some general chapters. The beginning chapters present definitions and concepts, historical matter, and practical laboratory matters, while the following chapters present theories, problems, difficulties, and controversies that confront the modern microbiologist. Thus two of the closing chapters are devoted to the problem of constancy of species.

It is a sane, well balanced, informative, and stimulating presentation of one of the most fascinating and practically significant aspects of biology.—G. K. K. LINK.

Grass manual

The Indiana Department of Conservation has again taken advantage of its opportunity by publishing another of DEAM'S books on Indiana plants. This volume[9] is a companion to the earlier publications of DEAM.[10]

The arrangement of the tribes and genera is that employed by HITCHCOCK in his *Genera of grasses in the United States*, which places the less specialized groups first. The treatment includes dichotomous keys to the genera of each tribe, while each genus has a key to species. Rather complete botanical descriptions have been drawn by the author from Indiana material. The general dis-

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[8] LUTMAN, B. F., Microbiology. pp. x+495. *figs. 211*. McGraw-Hill Book Co. New York. 1929.

[9] DEAM, C. C., Grasses of Indiana. Publ. 82. Dept. Conservation, State of Indiana. 1929.

[10] ———, Trees of Indiana. Publ. 13. Dept. Conservation, State of Indiana. 1921.
———, Shrubs of Indiana. Publ. 44. Dept. Conservation, State of Indiana. 1924.

tribution of species is described briefly. In addition the distribution of each species within the state is indicated by counties on separate maps by use of initials of the herbaria in which the specimens are deposited.

The excellent illustrations have been prepared by PAUL WEATHERWAX, who has also contributed an adequate account of the morphology of the grass plant. In each genus the habit of at least one species is illustrated, and the spikelet of each species is figured. The size and detail of the plates add considerable facility to the use of the manual, especially in view of the fact that the botanist usually avoids identification of grasses. The work recognizes 163 native species, 16 varieties, and 7 forms; 38 introduced species, and 3 varieties. For various reasons DEAM excludes 51 species that have been reported as occurring in Indiana.—S. A. CAIN.

NOTES FOR STUDENTS

Control of seeding behavior.—The causes of premature seeding of celery have been investigated by THOMPSON,[11] who finds that hardening of the seedling plants at low temperature (40–50° F.) is the main cause of this behavior. Freezing itself, popularly believed to induce first season blooming, was found to retard rather than to hasten it. Prolonged cold is particularly effective. With only 7–10 days of chilling the plants usually do not seed during the first summer, but remain biennial; with two weeks' treatment or longer, however, the plants are likely to show premature development of the reproductive phases of the life cycle, and become annuals. Hardening of the plants in the cold-frame by low temperature for too long a time is the most potent cause of commercial loss from this physiological disturbance.

Since the plants can be hardened effectively by water deficiency, without use of low temperatures, it is easy to prevent premature seeding. When late celery is seeded in outdoor beds, it may be induced to form seed stalks by cool weather during the seedling stages. (The reviewer has had the same experience with early sown turnips.) But high temperatures (70° F. or above) later in June and July can overcome this tendency, and prevent the premature development of blossoms.

High temperature treatment will prevent seeding, regardless of the previous treatment, provided differentiation has not proceeded too far before the high temperature is applied. Such facts as these account for the irregular behavior of celery under commercial cultural conditions. Early cold weather may be offset later by favorable high temperature conditions.

Naturally there are varietal differences in behavior. The rank-growing early sorts are more susceptible to climatic modification. The strains inherit certain capacities for development along vegetative and sexual lines. But the environmental conditions determine the exact nature of the response, developing vege-

[11] THOMPSON, H. C., Premature seeding of celery. Cornell Univ. Agric. Exp. Sta. Bull. 480. 1929.

tative or reproductive responses mainly through temperature modifications of metabolism.

As in many other such studies, chemical analyses of the plants do not help much in interpretation of the behavior. This is probably due to the fact that the effective metabolic conditions are centered in the differentiating region of the stem tip meristem, while our analyses are often made from the entire shoot. The differences in meristems may then be outweighed by the general average condition of the older tissues. Microchemical differences in the heart regions of these plants should be sought, and at the time differentiation is occurring, rather than after the differentiated plants have grown under the same conditions as the controls for a long period.

A study of seed stalk development of cabbage has been made by MILLER,[12] who also finds that temperature plays a large part in determining the vegetative and sexual responses of cabbage plants. Stalks of cabbage in head, removed to a warm greenhouse (60–70° F.), remained vegetative for two and one-half years, producing head after head, with intervening periods of stem elongation. After producing six successive heads on the same stem it was removed to a cool greenhouse, under which condition it blossomed in a few months. Heads given a rest period of two months in cold storage (40° F.) bloomed a month earlier in the cool greenhouse than those not given such a rest period. After such cold storage treatment, high temperature hastened seed development instead of inhibiting it. Cool treatment in the young stages of development was very effective in inducing seeding.

Chemical analyses of the older plants were made on samples from the heart region, and showed a definite accumulation of carbohydrates in the seeders. Analyses of the young plants showed little. Here again microchemical methods might be more effective.

As in the case of celery, it is a problem of both heredity and environment, a capacity to respond in various ways, and development of one response rather than another by the surrounding conditions, through some profound influence upon physiological processes and metabolism, particularly as these affect differentiation.

These papers are excellent contributions to our knowledge of plant behavior, and are helpful in pushing toward the goal of physiological research, controlled plant production.—C. A. SHULL.

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[12] MILLER, J. C., A study of some factors affecting seed-stalk development in cabbage. Cornell Univ. Agric. Exp. Sta. Bull. 488. 1929.

GENERAL INDEX

Classified entries will be found under Contributors and Reviews. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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